UNIVERSIDAD DE GUADALAJARA

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Efecto de extractos de algas marinas como promotores de crecimiento e inductores de resistencia en plantas de tomate (*Solanum lycopersicum*)

Tesis
que para obtener el grado de

Doctora en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas

Presenta

Rosalba Mireya Hernández Herrera

Las Agujas, Zapopan, Jalisco 17 de julio de 2013

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AGRADECIMIENTOS

A la Universidad de Guadalajara y en especial al Centro Universitario de Ciencias Biológicas y Agropecuarias, por el programa del Doctorado en Ciencias en Biosistemática, Ecología y Manejo de Recursos Naturales y Agrícolas (BEMARENA). A todos mis maestros del BEMARENA, por la educación, preparación y profesionalismo que mostraron a lo largo del proceso de aprendizaje en las aulas, laboratorios y campos experimentales.

Me gustaría agradecer a los miembros del comité particular por su gran apoyo y orientación para mi proyecto de tesis, Dr. Mario Alberto Ruiz López, Dr. Gil Virgen Calleros, Dr. Juan Francisco Zamora Natera por su asesoría, ayuda, orientación y consejos para la realización de este trabajo. También mi agradecimiento se extiende al Dr. Jeffrey Norrie y al Dr. Gustavo Hernández Carmona por compartirme sus conocimientos, su gran apoyo y sugerencias a lo largo de mi proyecto.

Mi más sincera gratitud ante todo, a la Dra. Carla Vanessa Sánchez Hernández, por su inmenso apoyo académico, orientación y estímulo a lo largo de mi proyecto de tesis. Sin su ayuda este proyecto de tesis no habría sido posible. Igualmente, me gustaría expresar mi agradecimiento especial al Dr. Fernando Santacruz Ruvalcaba por compartirme su gran amistad y su apoyo en la realización del presente trabajo.

A Emmanuel Furlong Estrada por su invaluable ayuda en el trabajo de campo, por su crítica constructiva en el escrito de la tesis y por compartir mis metas.

Doy las gracias a todos los miembros de los laboratorios que me apoyaron y brindaron su amistad durante la realización del presente trabajo: Laboratorio de Marcadores Moleculares (Dra. Isabel, Dra. Martha, Paulina, Martín y Alejandro), Laboratorio de Cultivo de tejidos (Lulú y Juve), Laboratorio Fitopatología (Carmen y Elizabeth), Laboratorio de Biotecnología (Lety Maya) y Laboratorio de Ficología (Adrian Ricardo).

A la Dra. Laura Guzmán Dávalos Coordinada del Doctorado BEMARENA, por su apoyo y facilidades otorgadas.

DEDICATORIAS

A Emmanuel, mi motivación, apoyo y compañero de vida

A mis hijas Andrea Aramis y Mildrett Josefina, por el amor que recibo de ellas todos los días

A mis padres (Conchita y Petro) y hermanas (Aurora, Angelica y Blanca) por su ejemplo de vida, trabajo, profesionalismo y bondad.

Quienes pueden, pueden porque piensan que pueden.

Virgilio

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ABREVIATURAS

PLA2	fosfolipasa, de sus siglas en inglés, phospholipase
AJ	ácido jasmónico
AS	ácido salicílico
AOS	sintasa de oxido de aleno, de sus siglas en inglés, allene oxide synthase
CYP2	cysteine proteinase de sus siglas en inglés, cistein proteasa
ET	etileno
HR	respuesta hipersensible de sus siglas en inglés, hypersensitive response
ISR	resistencia sistémica inducida de sus siglas en inglés, induced systemic resistance
JAMYC2	factor de transcripción MYC de sus siglas en inglés, MYC transcription factor
LOXD	lipoxigenasa de sus siglas en inglés, lipoxygenase
MeSA	jasmonato de metilo
PAL	fenilalanina amonio liasa de sus siglas en inglés, phenylalanine ammonia lyase
POD	polifenoloxidasas
PPO	peroxidasas
PIs	inhibidores de proteasas
PI-I	inhibidor de proteasa I de sus siglas en inglés, wound-induced proteinase inhibitor I
PI-II	inhibidor de la proteasa II de sus siglas en inglés, wound-induced proteinase inhibitor II
PR	proteínas relacionadas con patogénesis
SAR	resistencia sistémica adquirida de sus siglas en inglés, systemic acquired resistnace
SCP	serin carboxipeptidasa de sus siglas en inglés, wound-inducible carboxypeptidase
LSEs	liquid seaweed extracts

RESUMEN

Las algas marinas y los productos elaborados a base de ellas son utilizados alrededor del mundo para incrementar el crecimiento y rendimiento de las plantas, además les proporcionan protección ante agentes bióticos y abióticos. En México, existe poca información sobre el uso de las especies algales como fertilizante y no existe información alguna de los efectos como inductores de defensa sobre hongos fitopatógenos. El trabajo de investigación que aquí se presenta consta de dos partes y tienen en común el uso de extractos de algas marinas *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora* y *Sargassum liebmannii.* En un primer experimento se analizaron los compuestos minerales presentes en las algas marinas y se elaboraron extractos líquidos de algas, los cuales fueron empleados como bioestimulantes en la germinación y desarrollo de las plantas, los parámetros evaluados fueron porcentaje de germinación, índice de germinación, energía de germinación, tiempo medio de germinación, e índice de vigor de las plántulas), así como parámetros de crecimiento: longitud de plúmula y radícula, longitud de brote y raíz, el peso fresco y seco plantas de tomate (*Solanum lycopersicum*) en condiciones de laboratorio y en condiciones de invernadero aplicados directamente al sustrato o foliar a concentraciones de 0.2, 0.4 y 1.0 %. En un segundo experimento, a partir de los extractos líquidos se obtuvieron compuestos (carbohidratos) solubles. Estos extractos fueron liofilizados y utilizados como inductores de defensa en plantas de tomate. Los extractos se pesaron y diluyeron en agua estéril a una concentración de 0.1 mg mL^{-1} y se aplicaron sobre las hojas de las plantas hasta que el extracto escurrió. Para los ensayos de protección, una suspensión de (1×10^6 conidias mL^{-1}) de *A. solani* fue inoculada en las hojas. La severidad de la enfermedad fue evaluada 15 días después de la inoculación. Para los ensayos de proteína el material se colectó a las 24, 48 y 72 h posterior a la aplicación de los extractos e inmediatamente se congelo en nitrógeno líquido a –80°C. Se midió la actividad de proteínas relacionadas con defensa: polifenol oxidasas (PPO), peroxidasas (POD) e inhibidores de proteasas (PIs). Además se realizó un análisis de expresión de genes relacionados con defensa, sintasa óxido de aleno (*AOS*), lipoxigenasa (*LOXD*), factor de transcripción MYC (*JAMYC2*), fosfolipasa A2 (*PLA2*), serin carboxipeptidasa (*SCP*), fenilalanina amonio liasa (*PAL*), cistein-proteasa (*CYP2*), inhibidor de proteasa I (*PI-I*), e inhibidor de la proteasa II (*PI-II*).

Los resultados indican que las semillas que fueron embebidas con los extractos de *U. lactuca* y *P. gymnospora* a 0.2 % mostraron una mejor respuesta de germinación asociada con un tiempo medio de germinación menor un alto índice y energía de germinación y en consecuencia mayor vigor de las plántulas e incrementos en la longitud de las plántulas. Además se observó que los tratamientos empleados directamente al sustrato resultaron más eficaces en la longitud de la planta, la talla máxima registrada fue de 79 cm y las planta tratadas con aplicaciones foliares la talla fue de 75 cm. Además, Las plantas que recibieron los extractos de *U. lactuca* y *P. gymnospora* presentaron incrementos en la longitud del tallo, raíz y peso. También los extractos de *U. lactuca* y *P. gymnospora* resultaron mejores candidatos para elaborar bioestimulantes del crecimiento de plantas de tomate. Todos los extractos algales confirieron resistencia a *A. solani*, especialmente los extractos de *U. lactuca* y *P. gymnospora.* A partir de los resultados obtenidos de la actividad de las proteínas y ensayos de expresión genética que *U. lactuca* induce resistencia contra este hongo necrotrófico a través de la expresión de genes de respuesta sistémica a herida (SWRP), incluyendo defensa, vía de señalización y genes de proteasa. Se observaron correlaciones débiles entre la actividad enzimática, expresión de genes SWRP y la resistencia frente a *A. solani* con los extractos de *Caulerpa sertularioides*, *Padina gymnospora* y *Sargassum liebmannii.* Esto sugiere que otros mecanismos de defensa que el ácido jasmónico/sistemina de la vía de respuesta heridas son inducidos por polisacáridos de la pared celular presentes en los extractos de estas últimas tres algas. Este estudio proporciona información valiosa sobre la identificación y utilización de los recursos de algas marinas mexicanas para la agricultura. La presencia de minerales y carbohidratos en los extractos son una excelente opción como bioestimulantes o como inductores de resistencia. Por tanto, la práctica ecológica de la aplicación de extracto de algas marinas se puede recomendar a los productores para ayudar a lograr una mejor germinación y crecimiento de tomate o dc otras plantas.

ABSTRACT

Seaweed and products made from them are used around the world to increase the growth and yield of plants and also provide protection against biotic and abiotic agents. In Mexico, there is low information about the use of algal species as fertilizer and there is no information as elicitors of defense on fungal pathogens. The investigation presented here consists of two parts and have in common the use of seaweed extracts *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora* y *Sargassum liebmannii.* In a first experiment were analyzed mineral compounds present in the seaweed, and obtained liquid seaweed extracts, which were used as biostimulants on germination parameters (percentage, index, mean time, energy, and seedling vigor index) and growth parameters (plumule length, radical length, shoot length, root length, fresh weight and dry weight) of seedling tomato (*Solanum lycopersicum*) under laboratory and greenhouse conditions using foliar and soil drench applications of LSEs. In a second experiment, soluble compounds (carbohydrates) were obtained from liquid seaweed extracts (LSEs). These extracts were lyophilized and used as elicitor of defense in tomato plants. The extracts were weighed and diluted in sterile water at a concentration of 0.1 mg ml^{-1} and applied on the leaves of the plants until run-off or water (controls). For protection assay, a conidial suspension (1×10^6 conidia mL^{-1}) of *A. solani* was inoculated on a leaflet of six different leaves. Disease severity was evaluated 15 days after inoculation. For protein activity assays, leaf samples were collected at 0, 24, 48 and 72 h, after treatment and immediately frozen in liquid nitrogen and stored at –80°C. The activities of polyphenol oxidase (PPO), peroxidase (POD) and proteinase inhibitors (PIs) were measured. In addition, defense-related genes expression analysis by qPCR was performed, as allene oxide synthase (*AOS*), lipoxygenase (*LOXD*), MYC transcription factor (*JAMYC2*), phospholipase A2 (*PLA2*), wound-inducible carboxypeptidase (*SCP*), phenylalanine ammonia lyase (*PAL*), cysteine proteinase (*CYP2*), wound-induced proteinase inhibitor I (*PI-I*) and wound-induced proteinase inhibitor II (*PI-II*). Our results indicate that seeds treated with LSEs of *U. lactuca* and *P. gymnospora* at lower concentrations (0.2 %) showed enhanced for the germination (better response in germination rate associated with lower mean germination time, high germination index and germination energy and consequently greater seedling vigor and greater plumule and radicle length). Application as a soil drench was found to be more effective in influencing the height of the plant up to (79 cm) than the foliar spray application (75 cm). Plants receiving LSEs of *U. lactuca* and *P. gymnospora* showed more shoot, root length and

weight. Furthermore, *U. lactuca* and *P. gymnospora* were found to be more successful and the better candidates for developing effective biostimulants to improve the growth of tomato plants. All extracts were shown to confer resistance to *A. solani*, particularly those obtained from *U. lactuca* and *P. gymnospora*. It was evident from the protein activity and gene expression assays that *U. lactuca* induced resistance against this necrotrophic fungus through the expression of systemic wound response (SWRP) genes, including defense, signal pathway and protease genes. Increasingly weaker correlations between SWRP protein activity and gene expression and resistance against *A. solani* were observed for *C. sertularioides, P. gymnospora* and *S. liebmannii*. This suggests that defense mechanisms other than the jasmonic acid/systemin-wound response pathway are induced by cell wall polysaccharide preparations of the latter three algae. This study provides important information on the identification and utilization of Mexican seaweed resources for agriculture and is the first study to report on uses of these seaweeds as a source of liquid extracts as biostimulants and as elicitors in agriculture.

I. INTRODUCCIÓN

Actualmente se utilizan en diversos cultivos agrícolas y hortícolas sustancias bioactivas extraídas de las algas marinas como agentes de control biológico y/o biofertilizantes para mejorar su rendimiento y calidad.

En años recientes, los productos naturales a base de algas marinas se están utilizando como sustitutos de fertilizantes sintéticos y han adquirido importancia en varios países de todo el mundo. Estos productos se encuentran comercialmente como fertilizantes o bioestimulantes por su contenido de nitrógeno, fósforo, potasio y hormonas promotoras de crecimiento (auxinas y giberelinas), betainas, oligoelementos, vitaminas y aminoácidos (Khan et al. 2009).

El efecto estimulante de crecimiento los extractos de algas marinas en las plantas ya ha sido reportado y se ha demostrado que estos productos causan efectos benéficos en ellas, tales como mejoras en la movilización y distribución de nutrientes, estimulan la germinación de semillas, el crecimiento de planta en el desarrollo de raíces vigorosas, incremento del contenido de clorofila y área foliar, así como en el establecimiento de trasplantes. Además las plantas tratadas con extractos de algas muestran incremento en los rendimientos, calidad post cosecha y de almacenamiento del fruto, exhiben mayor resistencia a las plagas y enfermedades (Khan et al. 2009) (Figura 1).

Debido a sus efectos como protectores de plantas, se ha propuesto que los extractos de algas actúan como inductores de respuesta de defensa en las plantas (Cluzet et al. 2004). Sin embargo, su modo de acción aun no esta claro, lo que limita su utilización como productos benéficos.

Los mecanismos por los que las plantas responden a los patógenos y a los inductores con el fin de limitar la penetración y el crecimiento en sus tejidos están bien caracterizados. Las plantas se protegen contra patógenos por la percepción de moléculas señal llamadas inductores que incluyen una amplia variedad de moléculas tales como: oligo y polisacáridos, péptidos, proteínas, y lípidos, a menudo están presentes en la pared celular de los patógenos que atacan a las plantas (Boller 1995; Cote et al. 1998). Una variedad de polisacáridos presentes en extractos de algas son considerados como inductores eficaces de defensa de las plantas contra enfermedades (Vera et al. 2011).

Estudios previos han demostrado los efectos beneficos de la aplicación de extractos de algas marinas en las plantas, tales como germinación temprana, establecimiento de plántulas, mejoramiento y mayor rendimiento de los cultivos, incremento de resistencia a factores bióticos y abióticos (Kombrink y Somssich 1995) (**Figura 1**).

En México, recientemente debido a los efectos adversos que los fertilizantes químicos ocasionan al medio ambiente y la necesidad en obtener mayores rendimientos de los cultivos para satisfacer las necesidades alimentarias de los seres humanos, se está fomentando el estudio de nuevas fuentes naturales de fertilizantes, bioestimulantes e inductores de defensa, entre ellos los productos de algas marinas puede ser útiles para lograr una mayor producción de los cultivos.

Además, durante la última década, el control de las enfermedades de productos hortícolas es cada vez más difícil. A pesar de las grandes ventajas que han aportado al desarrollo de la agricultura, el uso excesivo de pesticidas ha afectado el medio ambiente y la salud humana. Aunado a esto, los procedimientos de reinscripción de fungicidas de amplio espectro y el aumento de la resistencia de cepas de hongos a fungicidas son algunos de los problemas principales que enfrentan los productores (Bruton 1994; De Waard et al. 1993).

Por todo lo anterior, el uso de extractos a base algas marinas ofrece la posibilidad de sustituir los agroquímicos y fungicidas por productos no agresivos al ambiente.

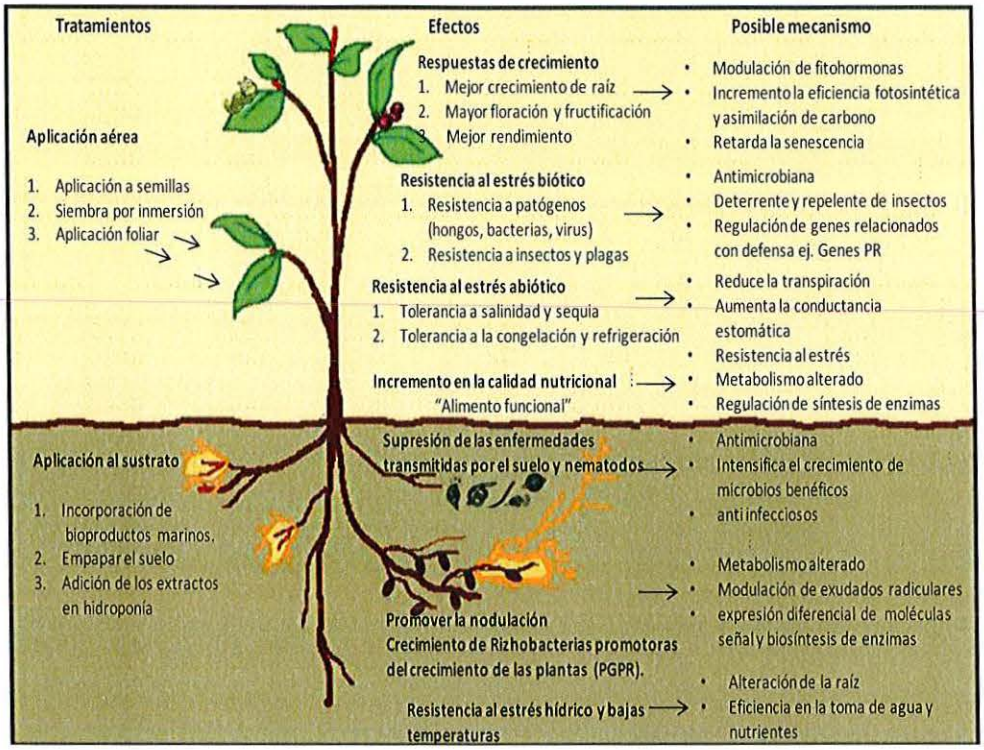

Figura 1. Representación esquemática de los efectos fisiológicos provocados por los productos a base de algas marinas y los posibles mecanismos de bioactividad. Imagen obtenida de Khan et al. (2009)

II. REVISIÓN DE LITERATURA

Este capítulo proporciona una visión general del estado actual de la investigación relacionada con los productos a base de algas marinas utilizados en la agricultura como fertilizantes o bioestimulantes; también se muestran los compuestos presentes en algas marinas responsables del crecimiento de las plantas y que son utilizados como inductores de defensa; se describe el patógeno examinado de interés *Alternaria solani*, así como la planta huésped tomate (*Solanum lycopersicum*) y los mecanismos de resistencia de las plantas hacia las enfermedades.

2.1. Productos elaborados a base de algas marinas utilizados en la agricultura

Una amplia investigación realizada por Chopin y Sawhney (2009) valora la industria de las algas a nivel mundial en cerca de 6 billones de dólares en el 2004 (Soto 2009). Los productos a base de algas se identifican y catalogan en tres subcomponentes principales: vegetales del mar, ficocoloides y ficosuplementos. Los productos relacionados con la agricultura están valuados en 50 millones de dólares y son representados como aditivos del suelo, agroquímicos (fertilizantes y bioestimulantes) y forrajes (suplementos, ingredientes) (**Cuadro 1**).

Los números indican una tasa de crecimiento relativamente lenta en los productos de agricultura, Jensen (1979) estimó la producción a nivel mundial de las algas como alimento en aproximadamente 10 millones de dólares (~30.000 ton/año, principalmente de *Ascophyllum nodosum*).

Cuadro 1. Valor a nivel mundial de la industria de las algas marinas en 2004

Productos	**Valor (US$, millones)**
Vegetales marinos	5,290
Ficocoloides	650[a]
Ficosuplementos	53
(Aditivos al sustrato)	(30)
(Agroquímicos)	(10)
(Alimento para animal)	(10)[b]
(Otros)	(3)
Total	**5,993**

Cuadro adaptado de Chopin and Sawhney (2009) [a]Bixler y Porse (2010), [b]Algas como alimento, usadas principalmente como suplemento, vitaminas y minerales. Producidas por varios tipos de kelps (*Ascophyllum nodosum*, *Fucus* spp., *Laminaria* spp. y *Macrocystis pyrifera*.).

El uso de las algas marinas con fines agrícolas ha sido documentado en 21 países de todo el mundo por Zemke-White y Ohno (1999) (**Figura 2**).

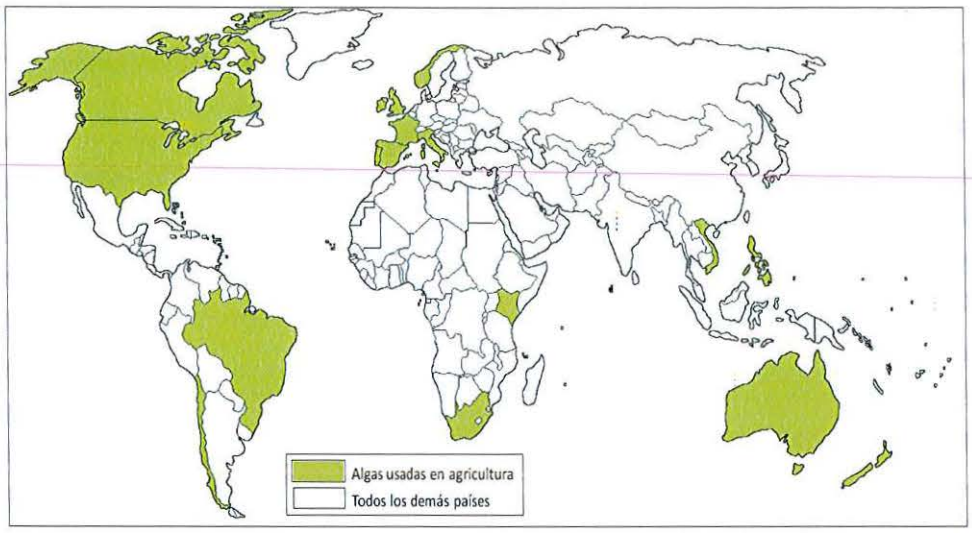

Figura 2. Países donde el uso de algas ha sido documentado. Adaptado de Zemke-White y Ohno (1999)

Recientemente México se incluye entre ellos. Existen tres empresas: Algas y Bioderivados Marinos S.A de C.V., Tecniprocesos Biológicos S.A de C.V. y Productos del Pacífico S.A de C.V., las cuales se dedican a elaborar productos que se utilizan en la agricultura y horticultura (Khan et al. 2009; SAGARPA 2012).

Inicialmente las algas marinas fueron comercializadas como productos secos y pulverizados e incorporados directamente al suelo, pero en los últimos años al menos 25 especies de algas marinas se utilizan como bioestimulantes, fertilizantes, mejoradores de suelo, estimulantes de crecimiento, productos saludables y alimento para animal a través de una amplia variedad de climas y sistemas de cultivo (Zemke-White y Ohno 1999) (**Cuadro 2**). Los extractos comerciales actuales, se fabrican principalmente de las algas marinas pardas *Laminaria digitata* (Lamouroux), *Fucus serratus* (L.), *Sargassum sp*, *Macrocystis pyrifera* (L.) C. Agardh, *Eckonia máxima* (Osbeck) Papenf., *Durvillea antarctica* (Chamisso) Hariot., y *Ascophyllum nodosus* (L.) siendo esta última especie la más utilizada (Norrie y Hiltz, 1999) y es cosechada en la costa de Canadá y Noruega. Actualmente otras especies de algas verdes como *Enteromorpha intestinalis* (L.) Link., *Ulva lactuca* (L.) y el alga roja *Kappaphycus alvarezii* (Doty) Doty, también se han utilizado (Gandhiyappan y Perumal 2001; Khan et al. 2009; Nabti et al. 2010; Rathore et al. 2009).

Cuadro 2. Productos comerciales de algas marinas utilizados en la agricultura y horticultura

Producto	Alga	Compañía	Aplicación
Acadian®	*Ascophyllum nodosum*	Acadian Agritech	ECP
Acid Buf	*Lithothamnium calcareum*	Chance & Hunt Limited	AA
Agri-Gro Ultra	*Ascophyllum nodosum*	Agri Gro Marketing Inc.	ECP
AgroKelp	*Macrocystis pyrifera*	Algas y Bioderivados Marinos, S.A. de C.V.	ECP
Alg-A-Mic	*Ascophyllum nodosum*	BioBizz Worldwide N.V.	ECP
Bio-Genesis™ High Tide™	*Ascophyllum nodosum*	Green Air Products, Inc.	ECP
Biovita	*Ascophyllum nodosum*	PI Industries Ltd	ECP
Emerald RMA	Alga roja marina	Dolphin Sea Vegetable Company	PS
Espoma	*Ascophyllum nodosum*	The Espoma Company	ECP
Fartum®	Inespecífico	Inversiones Patagonia S.A.	B
Guarantee®	*Ascophyllum nodosum*	Maine Stream Organics	ECP
Kelp Meal	*Ascophyllum nodosum*	Acadian Seaplants Ltd	ECP
Kelpak	*Ecklonia maxima*	BASF	ECP
Kelpro	*Macrocystis pyrifera*	Tecniprocesos Biológicos, S.A. de C.V.	ECP
Kelprosoil	*Macrocystis pyrifera*	Productos del Pacifico, S.A. de C.V.	ECP
Maxicrop	*Ascophyllum nodosum*	Maxicrop USA, Inc.	ECP
Nitrozime	*Ascophyllum nodosum*	Hydrodynamics International Inc.	ECP
Profert®	*Durvillaea antarctica*	BASF	BP
Sea Winner	Inespecífico	China Ocean University Product Development Co., Ltd	BP
Seanure	Inespecífico	Farmura Ltd.	ECP
Seasol®	*Durvillaea potatorum*	Seasol International Pty Ltd	ECP
Soluble Seaweed Extract	*Ascophyllum nodosum*	Technaflora Plant Products, LTD	ECP
Stimplex®	*Ascophyllum nodosum*	Acadian Agritech	ECP
Synergy	*Ascophyllum nodosum*	Green Air Products, Inc.	ECP
Tasco®	*Ascophyllum nodosum*	Acadian Agritech	AA

ECP = Estimula el crecimiento de las plantas; AA = Alimento de animales, BP = Bioestimulante de plantas, B = Biofertilizante PS = Producto saludable

Estos productos son fabricados a base de distintas especies de algas deshidratadas o partes frescas de ellas, y son procesados utilizando diferentes métodos de extracción, los extractos en el mercado actual son preparaciones acuosas que varían en color desde casi incoloro a un intenso negro-marrón oscuro. Del mismo modo, los olores, viscosidades, sólidos y

partículas contenidos de materia son muy variables. En general, los extractos se hacen por procesos que utilizan agua, álcalis o ácidos, o físicamente por molienda utilizando vapor y presión (Hervé y Rouillier 1977).

Actualmente, existen métodos de extracción que producen un concentrado sin tener que acudir a un tratamiento químico o con calor, el material es sometido a un cambio rápido de presión que rompe los componentes estructurales de las células permitiendo liberar prácticamente todos los reguladores de crecimiento del alga (RCPs), en cambio las harinas son un polvo grueso del la misma especie de alga seca molida y por lo tanto tienen ciertas cualidades comunes a los extractos líquidos (Bula-Meyer 2004; Stirk y van Staden 1997).

Los extractos de algas marinas son bioactivos en concentraciones bajas (Dilución 1:1000 o más) (Crouch y van Staden 1993). Aunque muchos de los diversos componentes químicos y de sus modos de acción siguen siendo desconocido, es posible que estos componentes presenten actividad sinérgica (Fornes et al. 2002; Vernieri et al. 2005). Debido a que la cantidad de nutrientes esenciales requeridos por las plantas es mas alto, las concentraciones que suministran con las algas marinas y con los extractos no cumplen con la definición legal de un fertilizante, por lo tanto, los productos de las algas marinas se comercializan como bioestimulantes o mejoradores del suelo.

Los bioestimulantes son capaces de incrementar el desarrollo, la producción y/o crecimiento de los vegetales. Russo y Berlyn (1990) los definen como productos no nutricionales que pueden reducir el uso de fertilizantes y aumentar la producción y la resistencia al estrés causado por la temperatura y déficit hídrico. Los biestimulantes no pueden llegar a suplir todos los nutrientes esenciales en las cantidades que una planta requiere (Schmidt et al. 2003), sin embargo su principal función es incrementar la absorción de minerales por la planta, haciendo más eficaz el uso de los nutrientes en las raíces y hojas.

Recientemente los extractos de algas marinas tienden a tener más éxito que los fertilizantes químicos ya que son considerados como un producto ecológico que no tiene efectos adversos como los abonos y agroquímicos sintéticos que dañan el medio ambiente (Thirumaran et al. 2009) y por su aplicación en bajas dosis es factible usarlos en áreas distantes al mar (Senn 1987).

2.2. Compuestos presentes en algas marinas que afectan el crecimiento de las plantas

Se ha reportado que el efecto del uso de algas marinas y/o sus derivados en la agricultura se debe a que contienen macro y microelementos, se han reportado aproximadamente 56 elementos presentes en las algas marinas (Vinogradov 1953). Los macroelementos identificados en las algas marinas incluyen (C, H, O, K, N, S, P, Ca y Mg) y estan presentes generalmente en concentraciones superiores a 1 mg por gramo de peso seco (Booth 1965; Stephenson 1968). Así mismo los microelementos identificados en las algas incluyen Fe, Cu, Zn, Mn, Si, I, Br y Na (Black y Mitchell 1952; Booth 1965; Bryan 1969; Yamamoto y Ishibasi 1972).

Tambien presentan una diversa gama de compuestos orgánicos, proteínas y aminoácidos y una gran variedad de vitaminas B, B2, B12, C, D3, E, K, ácido pantoténico, fólico y folínico, así como la presencia del precursor de la vitamina A, el ß-caroteno y otros posibles precursores (Hong et al. 2007; Stephenson 1968).

Además, las algas contienen sustancias como citocininas, auxinas y ácido abscísico, tipo (ABA) que tienen actividad como reguladores de crecimiento y actúan sobre el desarrollo y metabolismo celular en las plantas tratadas, lo cual conduce a un mejor crecimiento y rendimiento de los cultivos (Booth 1966; Crouch y van Staden 1992 y 1993; Durand et al. 2003; Ördög et al. 2004; Stirk et al. 2003). Estos compuestos presentes en las algas suelen ser muy eficaces a bajas concentraciones (Brain et al. 1973).

Los carbohidratos como el xilano presente en las algas rojas y verdes, los mananos y ulvanos característicos de las algas verdes y el ácido algínico, laminarina y el manitol de las algas pardas, se encuentran en las preparaciones comerciales que son utilizadas como fertilizante (Bula-Meyer 2004).

2.3. Compuestos presentes en las algas marinas que son utilizados como inductores de defensa en las plantas

Las plantas interactúan con el medio ambiente mediante la detección de moléculas nombradas inductores derivados de patógenos u otras fuentes. Estas moléculas se unen a receptores específicos localizados en la membrana plasmática y desencadenan respuestas de defensa que conducen a la protección contra los agentes patógenos.

En particular, se ha demostrado que los polisacáridos de la pared celular (ulvanos, alginatos, fucanos, laminarina y carragenanos) presentes en macroalgas marinas verdes, pardas y rojas pueden desencadenar respuestas de defensa en las plantas incrementando de la protección contra los patógenos. Además, los oligosacáridos obtenidos por despolimerización de los polisacáridos de algas marinas también inducen una protección contra las infecciones virales, fúngicas y bacterianas en plantas.

La activación de estas vías de señalización conduce a un incremento en la expresión de genes relacionados con la patogénesis (PR), proteínas con actividad antifúngica y antibacteriana, enzimas de defensa como fenilalanina amonio liasa (PAL) y lipoxigenasa (LOX) que determinan la acumulación de compuestos fenilpropanoides (PPC) y oxipilinas con actividad antiviral, antibacteriana y antifungica, síntesis de terpenos, terpenoides y/o alcaloides que tienen actividades antimicrobianas.

Por lo tanto, los polisacáridos y oligosacáridos derivados de las macroalgas inducen la acumulación de proteínas y compuestos con actividad antimicrobiana que determinan, al menos en parte, un mejoramiento en la protección contra los agentes patógenos en plantas (Vera et al. 2011).

2.4. El patosistema tomate – *Alternaria solani*

Taxonómicamente el tomate pertenece a la familia de las Solanáceas y su nombre científico es *Solanum lycopersicum* (Linneo 1753). Es originario de América del Sur, aunque se le considera a México como centro de su domesticación. Se trata de uno de los cultivos de mayor interés comercial a escala global. Es una planta cultivada anualmente sobre diferentes tipos de suelo y climas, su cultivo en invernadero y riego hidropónico ha favorecido su producción continua a lo largo de todo el año.

Actualmente forma parte de la dieta alimenticia de varias culturas en todo el mundo. El fruto del tomate contiene baja cantidad de calorías, alto contenido de fibra, minerales, vitaminas y fenoles tales como flavonoides hacen del tomate un excelente vegetal proporcionando beneficios nutricionales al consumo humano (Dorais et al. 2008).

Ya sea como hortaliza o como fruto, el tomate ocupa en nuestros días el segundo lugar en importancia entre los productos agropecuarios, apenas aventajado después de la papa, con una producción anual de 122.9 millones de toneladas de fruto fresco (FAO 2005). En 2009 la producción de tomate en México fue casi 3 millones de toneladas (FAO 2009). Sin embargo uno de los principales problemas que afectan la producción de tomate en México es la intensa aplicación de fertilizantes químicos, causando daño al estado ecológico de los sistemas agrícolas (Villarreal-Sánchez et al. 2003).

El tizón temprano del tomate, es causado por *Alternaria solani* (Ellis y Martin) Jones y Grout, es la enfermedad más importante del cultivo de tomate en EE.UU., Reino Unido, Australia, Israel, India y México, donde las reducciones significativas en el rendimiento son del (35 a 78 %) (Datar y Mayeen 1972; Jones et al. 1993). Los síntomas característicos de la enfermedad en hojas son manchas circulares o angulares de hasta 0.5 cm de diámetro, de color pardo con marcados anillos concéntricos solitarias o en gran número sobre las hojas. Las áreas afectadas se tornan amarillas, luego pardas y las hojas cuelgan. Este manchado comienza por las hojas más viejas y luego prospera hacia arriba, la planta entera puede defoliarse y morir. En el tallo y peciolo produce lesiones negras alargadas, en las que se puede observar a veces anillos concéntricos. Los frutos son atacados a partir de las cicatrices del cáliz, provocando lesiones pardo-oscuras ligeramente aplastadas y recubiertas de numerosas esporas de hongo (Gaber y Wiebe 1997) (**Figura 3**).

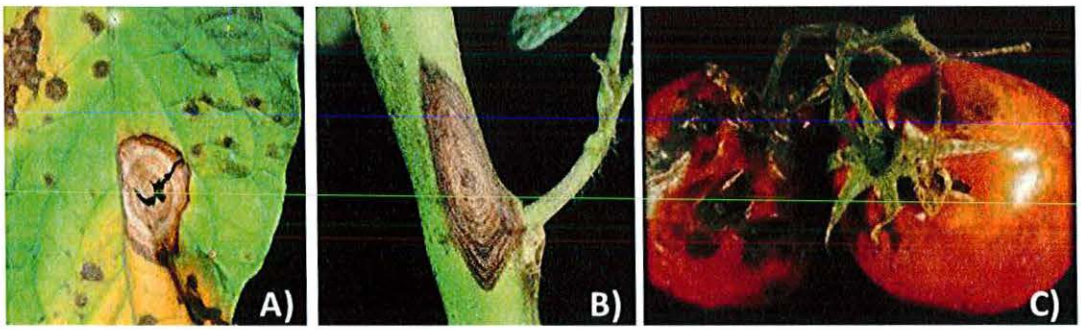

Figura 3. Planta de tomate A) hoja, B) tallo y C) fruto con los típicos síntomas de *Alternaria solani.* Las manchas necróticas marrones oscuras tienen anillos concéntricos en los donde las nuevas esporas se producen y están rodeadas por un halo clorótico causado por las toxinas secretadas

La infección de las plantas puede resultar en una pérdida total de la cosecha, los rendimientos de la planta se reducen por la destrucción de follaje y los frutos se dañan directamente por el patógeno y por manchas de sol en plantas defoliadas (Rotem 1994). La enfermedad debilita progresivamente a la planta y aumenta su susceptibilidad a la infección por la reducción del área fotosintética de la hoja y aumentar el desequilibrio entre la demanda de nutrientes en los frutos y el suministro de nutrientes de las hojas (Rowell 1953).

La germinación de las conidias y la penetración del hongo pueden ocurrir bajo una amplia gama de temperaturas, entre 4 y 36 °C (Pound 1951) y sólo requiere un breve período húmedo de al menos cuatro horas para una infección exitosa (Vloutoglou y Kalogerakis 2000). Típicamente, los tejidos debilitados de la planta, ya sea debido a la tensión, la senescencia, o heridas, son más susceptibles a la infección por *Alternaria* que los tejidos sanos (Thomma 2003).

Alternaria es un patógeno necrotrófico, es decir, que el hongo invasor mata a las células vegetales para después extraer los nutrientes de la célula, en lugar de desarrollar haustorios y mantener el tejido de la planta viva como lo hacen los patógenos biotróficos. Su micelio es oscuro, septado, ramificado; los conidióforos son cortos, oscuros y también septados. Las conidias son claviformes, oscuras, con septos transversales y longitudinales (Romero-Cova 1988) (**Figura 4**). *Alternaria* no tiene ninguna etapa conocida sexual o esporas de hibernación, pero el hongo puede sobrevivir como micelios o conidias en los restos vegetales en descomposición durante un período de tiempo considerable, o como una infección latente en las semillas (Rotem 1994).

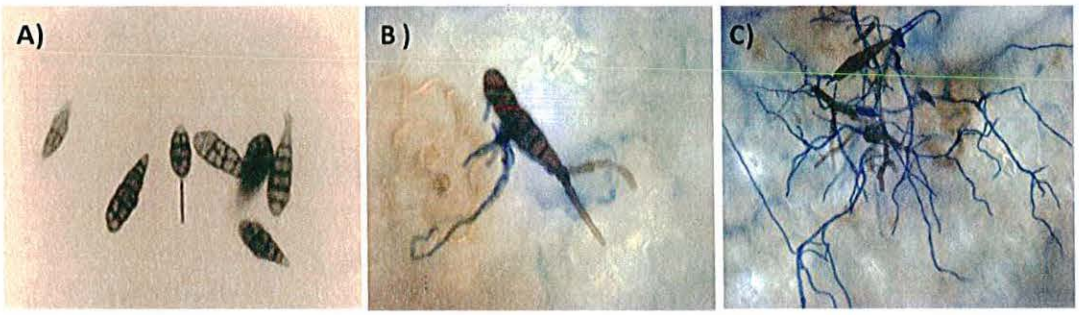

Figura 4. A) Conidias de *A. solani*, B) Germinación de conidias de *A. solani* y células epidérmicas muertas (izquierda de color marrón) por tóxinas en la superficie de la hoja de tomate antes de la penetración del hongo C) dos conidias y ramificación del micelio (color azul).

Bajo condiciones favorables los conidias de *Alternaria* germinan en horas y pueden producir más de un tubo germinal. La capacidad de penetrar en la cutícula, estomas, y las heridas han sido descritas para la mayoría de las especies de *Alternaria* (Rotem 1994). En el caso de las plantas jóvenes o resistentes a las cepas menos virulentas, la germinación de las hifas de *A. cassiae* y *A. alternata* tienden a extenderse sobre la superficie de la hoja intacta y los únicos sitios de infección son células muertas, lo que sugiere que la penetración está condicionada por la secreción de toxinas (van Dyke y Trigiano 1987; von Ramm 1962).

2.5. Mecanismos de resistencia de plantas hacia enfermedades

A continuación se explica de manera general como se inducen las respuestas de resistencia de las plantas hacia los patógenos.

2.5.1. La resistencia en plantas

Las plantas son atacadas continuamente por diverssos patógenos pero también son resistentes a la mayoría de ellos. Debido a un sistema sofisticado de defensa. Sin embargo, los patógenos han sido capaces de desarrollar mecanismos para suprimir o vencer el sistema de defensa de un hospedero. Hongos, bacteria y virus requieren al menos de ciertos estados de su ciclo de vida, de células hospederas para reproducirse (biotropos obligados, biotropos o hemibiotropos), donde algunos de ellos usan enzimas tóxicas o metabolitos para matar o vivir en las células muertas (necrotrófos) (Custers 2007a).

Las plantas emplean diferentes líneas de defensa. Una primera línea pasiva es la cutícula cerosa y la pared celular con la cual previene la entrada de muchos patógenos y sirve de protección contra la desecación. Varios virus, bacteria y hongos patógenos son incapaces de atravesar esta capa y solo pueden hacerlo a través de heridas o aperturas naturales como estomas. Células subyacentes a la pared celular, también son una barrera que puede evitar la entrada de patógenos a la planta. En cambio, los patógenos producen enzimas como: poligalacturonasas, celulasas y xilasas, que disuelven la pared celular de las plantas. Estas enzimas son predominantemente expresadas durante la infección y frecuentemente requeridas para la virulencia. Las celulasa sintetasa, por ejemplo, produce la celulosa para el ensamble de las

células en las plantas. Las mutaciones en los genes de dicha enzima pueden reducir el nivel de celulasa y estimular la formación de lignina como otra respuesta de defensa (Custers 2007a).

Aun cuando el patógeno rompe mecánicamente dichas barreras de defensa, muchas plantas presentan cantidades significativas de compuestos antimicrobianos tales como: fenoles, glucósidos, lactosas insaturadas, compuestos sulfurados, saponinas, glicósidos cianogélnicos, glucosinolatos alcaloides y polifenoles. Estos son liberados en la planta por lisis de vacuolas. En algunos casos los precursores son activados por síntesis de novo de enzimas vegetales llamadas fitoantocipinas. En contraste, fitoalexinas son sintetizadas en respuesta del ataque por patógenos. Un par de ellas, las saponinas de (avenacina) y de tomate (α tomatina) han sido estudiadas en mayor detalle (Custers 2007a).

2.5.2. La resistencia sistémica adquirida

Otra forma para la defensa de las plantas se da mediante la inducción de resistencia, que es el incremento de la capacidad de defensa en la planta por estimulación apropiada. La inducción de resistencia, es la activación de mecanismos de defensa que se expresa cuando la planta es desafiada por algún patógeno. El estímulo de resistencia puede ser desencadenado por ciertos químicos y patógenos avirulentos o virulentos. Generalmente la inducción de resistencia es sistémica, debido a que la capacidad defensiva se incrementa, no solo donde ocurre el contacto con el agente inductor, sino que se extiende a áreas distales. Por este carácter sistémico, generalmente esta inducción de resistencia se denomina resistencia sistémica adquirida (SAR). Sin embargo, la inducción de resistencia también ocurre de manera localizada cuando el tejido se expone a una primera invasión, donde se genera mayor resistencia. Ahora se sabe que la SAR es activada en varias especies de plantas y puede generar necrosis como parte de la respuesta de hipersensibilidad o como síntoma de la enfermedad. La resistencia conferida puede ser duradera, algunas veces por el ciclo de vida de la planta, y efectiva en un amplio espectro de patógenos incluyendo virus, bacterias y hongos.

La SAR es caracterizada por una acumulación de ácido salicílico (AS) y por la expresión de proteínas relacionadas con la patogénesis (PRs) (van Loon et al. 1998). Estas proteínas fueron descritas por van Loon (1970), quien observó la acumulación de varias proteínas después de la infección de plantas de tabaco con el virus del mosaico del tabaco (VMT). Cabe señalar que,

aunque *in vitro* varias de estas proteínas presentan propiedades antimicrobianas, la función en la respuesta de defensa aun no está claramente definida. En algunos trabajos como los de White (1979), se demostró la acumulación de PRs y generación de resistencia en plantas de tabaco hacia el VMT por la aplicación de ácido salicílico (AS) o ácido benzoico. Hasta hace pocos años, la identificación de genes reguladores para la defensa en plantas, generó evidencia de que las plantas usan varios mecanismos de defensa para evadir diferentes patógenos. Los mecanismos de defensas son caracterizados por las moléculas de señalización cruciales para la regulación de expresión de proteínas de defensa (Custers 2007b).

Como se menciono anteriormente, los patógenos son capaces de evadir varias capas de defensa, mientras la planta puede responder encendiendo mecanismos de defensa que proven resistencia hacia virus, bacterias y hongos, nematodos e insectos. Hasta ahora han sido identificados tres mecanismos de defensa, los dependientes del ácido salicílico (AS), los del ácido jasmónico (AJ) y del etileno (ET). El mecanismo dependiente del AS puede ser inducido por patógenos necrotrófos induciendo resistencia sistémica adquirida (SAR) que provee protección en contra de un amplio rango de estos. El mecanismo dependiente del AJ/ET provee resistencia contra hongos necrotróficos e insectos. Un tercer mecanismo que es también dependiente del AJ y ET puede ser inducido por rizobacterias no patógenas, provocando una resistencia sistémica inducida (ISR) en la planta (Custers 2007b). Hacia una comprensión molecular de las vías de resistencia sistémica existen dos rutas que han llamado la atención de los investigadores principalmente, donde involucra la participación de ácido salicílico (AS) o de ácido jasmónico (AJ)/ET como compuestos en clave de señalización. En ambas rutas resultan acumulación de productos de diferentes genes de defensa (Dong, 1998; Reymond y Farmer, 1998).

La proteína más común descrita como marcador para la ruta del ácido salicílico es la PR-1, mientras que PDF1.2 y los genes inhibidores de la proteasa son correlacionados con las vías del AJ/ET. La expresión de los genes que codifican las proteínas PR básicas que están generalmente localizadas en vacuola son frecuentemente atribuidas a la defensa regulada por la ruta del AJ/ET (van Loon 1997). En contraste las PRs ácidas se piensa que están en el espacio extracelular (apoplasto) y están asociados con la regulación del AS en esta ruta.

La activación de la ruta regulada por el AS se asocia con eventos que causan la necrosis. Así pues, la ruta se ajusta a la respuesta hipersensible (HR) donde la muerte celular programada de la planta es parte del mecanismo por el cual un agente patógeno es contrarrestado en el sitio inicial de la invasión. La muerte celular por HR inicia eventos de resistencia, denominada resistencia local, en las células que rodea el sitio de contención (Dangl et al. 1996). Con el tiempo la expresión de genes de defensa ocurren a gran distancia dando como resultado un efecto sistémico (Epple et al. 2003). Los patógenos que causan necrosis como parte de su sintomatología también inducen la ruta regulada por el AS.

2.5.3. Mecanismo de resistencia dependiente del ácido salicílico (AS)

La señalización del AS es esencial para SAR, para la iniciación de la respuesta de defensa local y para alguna interacción gen por gen. La SAR se inicia cundo la planta es desafiada por el patógeno que induce la necrosis local (Custers 2007a). La limitación del incremento del nivel del AS en hojas y en el floema, condujo a que muchas investigaciones se desarrollan en torno a que el AS pudiera ser la señal sistémica para la SAR. Un estudio reciente, sugiere que la señalización puede ocurrir a través de la conversión de AS a compuestos volátiles, como metil salicilato, el cual puede inducir resistencia a plantas no solo en partes donde se inoculó, sino también en plantas vecinas (Heil y Ton 2008). Probablemente, el papel del AS es restringido a la señalización local. Con los experimentos de Vernooij et al. (1994) se demostró que el AS no es la señal móvil para el desarrollo de SAR. Park et al. 2007 en su trabajo realizado con injertos de mutantes, presentaron pruebas de que jasmonato de metilo (MeSA), en lugar de AS, funciona como la señal crítica móvil. El AS se puede presentar en la planta en dos formas libre que probablemente tenga una función de señalización y en forma de almacenamiento, acido β-O-D-glucosalicílico (AGS) probablemente inactivo en la señalización. De esta forma, la conversión de AGS a AS libre y AS activo, puede influenciar fuertemente en la señalización del AS que además depende del tipo de la planta ya que diferentes especies presentan diversas cantidades de AS endógeno.

2.5.4. Mecanismo de resistencia dependiente del ácido jasmónico y etilieno(AJ/ET)

El ácido jasmónico (AJ) y etileno (ET), con frecuencia son requeridos simultáneamente para la resistencia en plantas en contra de patógenos específicos. Esto se demostró por el requerimiento para la expresión de genes de defensa en ambas hormonas de la planta. El AJ y análogos, también participan en la producción de polen y en la respuesta de heridas. Un número de metabolitos secundarios asociados con defensa que son también inducidos por AJ incluyen genes de proteína, cuyos productos exhiben actividad antimicrobiana y antimicótica, tales como la proteína defensina1.2 (PDF1.2) y Tionina2.1 (THI2.1) y se acumulan en respuesta a patógenos necrotrófos (Rojo et al. 2003). Además, en plantas de tomate (*Solanum lycopersicum*), infestadas con orugas, la señalización del AJ es importante en la regulación de los genes de respuesta a la herida, como los Inhibidores de proteasas, Polifenoloxidasas, Deaminasa treonina y Arginasa, las cuales tienen funciones antagonistas en el crecimiento y el desarrollo de la oruga (Chen et al. 2005; Ryan 2000). Además, el AJ regula la proteína vegetativa de almacenamiento (VSP1). Además, junto con las defensas directas, el AJ/ET induce vía indirecta defensas a través de la producción y liberación de compuestos volátiles (VOCs), así como volátiles de hoja verde (GLVs) o néctar extra floral de plantas que atraen tanto a depredadores y parasitoides de los herbívoros (Kessler y Baldwin 2002). Aunque algunos compuestos volátiles tienen efectos directos en contra de los herbívoros y patógenos, esta estrategia se conoce generalmente como "defensa indirecta". Por lo tanto, la activación indirecta de defensas también implica la regulación de las señales a larga distancia, en este caso el jasmonato de metilo (MeJA) es la molécula crucial en este tipo de resistencia. El ET, siguiente en el rol de defensa hacia patógenos, participa en varios procesos fisiológicos como la maduración de frutos y senescencia. Ambas hormonas son requeridas para el desarrollo de ISR y forma parte de la resistencia hacia insectos y hongos patógenos (Custers 2007a).

El uso de mutantes que se encuentran afectadas tanto en la acumulación como en la respuesta al AS, muestra una expresión elevada de genes regulados por AJ/ET, que en ocasiones puede ser revertida por la adición de un inductor de la señalización.

2.5.5. Genes de resistencia y la respuesta de hipersensibilidad (RH)

La reacción o respuesta de hipersensibilidad, es el sistema de defensa más poderoso que tienen las plantas. Es una respuesta compleja de defensa altamente concentrada (temporal y espacialmente) que genera muerte de células, alta acumulación local de compuestos fenólicos y reforzamiento de la pared celular en células que están alrededor de las células muertas. Además de la inducción de defensa general, lo cual previene una futura infección en partes distales de la planta. Aunque la respuesta de defensa ha sido exitosa y puede parar la infección por virus, nematodos, bacterias y hongos, es limitada, pues normalmente se desencadena por el alto reconocimiento específico, a través de gen de resistencia de una molécula inductora (asociado al patógeno) (Custers 2007b).

2.5.6. Resistencia específica (gen-por gen)

La resistencia gen-por-gen fue originalmente descrita en la década de los 40's por Flor (1946) quien estudio la genética de la interacción entre lino y el hongo *Melampsora lini*, observando que por cada gen de resistencia dominante en la planta, se presentaba un gen dominante avirulento en el hongo. La definición inicial de genes avirulentos de patógenos implico que se tuviera la habilidad de inducir resistencia en el hospedero que llevara los genes complementarios de resistencia. Presumiblemente, proteínas avirulentas unidas a la planta, desencadenan diferentes formas de proteína-R. Productos de genes de resistencia pueden participar en la protección de objetivos viruentos, esta modificación sigue la iniciación de defensa en plantas (Clusters 2007a). Ahora se ha propuesto una alternativa bioquímica de interpretación de resistencia de gen-por-gen y es la llamada "Modelo guardian", la cual postula que la proteína-R (el guardián) detecta cambios desencadenados por proteína AVR en una planta como blanco para la virulencia (Mackey et al. 2002).

III. HIPÓTESIS

Los extractos de algas marinas poseen compuestos con actividad biológica que favorecen la germinación, el crecimiento y promueven resistencia endógena de las plantas de tomate (*Solanum lycopersicum* L.)

IV. OBJETIVOS

General

Evaluar el efecto de extractos de algas marinas como promotores de crecimiento e inductores de resistencia en plantas de tomate (*Solanum lycopersicum* L).

Particulares

1. Analizar la composición química de las algas marinas de las especies *Ulva lactuca, Caulerpa sertularioides, Padina gymnospora* y *Sargassum liebmannii.*

2. Elaborar extractos acuosos y etanólicos de las especies *U. lactuca, C. sertularioides, P. gymnospora* y *S. liebmannii.*

3. Evaluar el efecto de extractos acuosos de algas marinas en la germinación y el crecimiento de plántulas de tomate en condiciones de laboratorio.

4. Evaluar el efecto de extractos acuosos de las algas marinas en el crecimiento de plantas de tomate en invernadero mediante aplicaciones foliares y al sustrato.

5. Evaluar la resistencia y/o severidad del tizón temprano en plantas de tomate (*S. lycopersicum*) expuestas a tratamiento foliar con extractos etanólicos de algas marinas.

6. Determinar la actividad de proteínas asociada a defensa: polifenol oxidasas (PPO), peroxidasas (POD), e inhibidores de proteasas (PIs) en plantas de tomate tratadas con extractos etanólicos.

7. Determinar la expresión de genes relacionados con defensa en plantas de tomate expuestas a tratamientos foliares con extractos etanólicos.

V. EXTRACTOS LÍQUIDOS DE ALGAS MARINAS EN EL CRECIMIENTO

JmlID 10811_ArtID 78_Proof# 1 - 04/07/2013

J Appl Phycol
DOI 10.1007/s10811-013-0078-4

Effect of liquid seaweed extracts on growth of tomato seedlings (*Solanum lycopersicum* L.)

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Received: 12 March 2013 / Revised: 26 June 2013 / Accepted: 27 June 2013

Abstract Seaweed extracts are used as nutrient supplements, biostimulants, or biofertilizers in agriculture and horticulture to increase plant growth and yield. In this study, we examined the effect of liquid seaweed extracts (LSEs) made from *Ulva lactuca*, *Caulerpa sertularioides*, *Padina gymnospora*, and *Sargassum liebmannii* as biostimulants on the germination and growth of tomato (*Solanum lycopersicum*) under laboratory and greenhouse conditions using foliar and soil drench applications of LSEs. We assessed LSEs at different concentrations (0.2, 0.4, and 1.0 %) on germination parameters (percentage, index, mean time, energy, and seedling vigor index) and growth parameters (plumule length, radical length, shoot length, root length, fresh weight, and dry weight) of tomato seedlings. Our results indicate that seeds treated with LSEs of *U. lactuca* and *P. gymnospora* at lower concentrations (0.2 %) showed enhanced germination (better response in germination rate associated with lower mean germination time, high germination index, and germination energy, and consequently greater seedling vigor and greater plumule and radicle length). Application as a soil drench was found to be more effective in influencing the height of the plant (up to 79 cm) than the foliar spray application (75 cm). Plants receiving LSEs of *U. lactuca* and *P. gymnospora* showed increased shoot length, root length, and weight. Furthermore, *U. lactuca* and *P. gymnospora* were found to be more successful and better candidates for developing effective biostimulants to improve the growth of tomato plants. This study provides important information on the identification and utilization of Mexican seaweed resources for agriculture and is the first study to report on the uses of these seaweeds as a source of liquid extracts as biostimulants in agriculture.

Keywords Seed germination · Seaweeds · Extract · Nutrient analysis · Biofertilizer · Chlorophyta · Phaeophyta

Introduction

Seaweed and seaweed products have been used worldwide to increase plant growth and yield. Modern agriculture is searching for new biotechnologies that would allow for a reduction in the use of chemical inputs without negatively affecting crop yield or the farmers' income. In recent years, the use of natural seaweed as fertilizer has allowed for partial substitution of conventional synthetic fertilizer (Dhargalkar and Pereira 2005; Hong et al. 2007; Khan et al. 2009; Zodape et al. 2010). In addition, a number of commercial seaweed extract products are available for use in agriculture and horticulture and can be used as liquid extracts applied as foliar spray, soil drench, or in granular/powder form as soil conditioners and manure (Blunden et al. 1997; Lingakumar et al. 2004; Thirumaran et al. 2009). These extracts are marketed as liquid biostimulants because a chemical analysis of seaweeds and their extracts has revealed the presence of a wide variety of plant growth-promoting substances such as auxins, cytokinins, and betaines (Khan et al. 2009). These substances can influence shoot and root system development (Durand et al. 2003; Stirk

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et al. 2004). As well, macronutrients and micronutrients can help promote the growth of various vegetables, fruits, and other crops (Blunden 1991; Crouch and van Staden 1993; Moller and Smith 1998). Many beneficial effects have been reported on the use of seaweed extracts. Positive responses include improved germination, root development, leaf quality, general plant vigor, and resistance to pathogens (Khan et al. 2009).

Seaweed is an inexpensive local resource along coastal agricultural areas. In Mexico, seaweeds are abundantly available and represent a great potential for eventual commercial exploitation (Gojón-Báez et al. 1998). However, the only commercial extracts currently produced in Mexico from raw seaweeds are Kelpro and Kelprosoil from *Macrocystis pyrifera* (SAGARPA 2012) and Algaenzims from *Sargassum* spp. (Canales-López 2000; Sunarpi et al. 2010). These products are available for use in agriculture and horticulture. One positive step towards the inclusion of native seaweed resources in Mexico is to use biofertilizers derived from seaweed as an alternative input to improve negative cropping conditions such as the progressive degradation of ecosystems and the contamination of agricultural lands caused by synthetic fertilizers.

Tomatoes (*Solanum lycopersicum*) are one of the most important vegetable crops around the world in terms of human consumption, and they are also the most popular garden vegetable. In 2009, Mexico was among the top ten tomato-producing countries contributing with almost three million tonnes (FAOSTAT 2009) to the global production. However, one of the main problems facing tomato production in Mexico is the intensive application of chemical fertilizers causing damage to the soil ecology and agricultural systems (Villarreal-Sánchez et al. 2003). The aim of this study was to examine the effect of liquid seaweed extracts derived from Mexican resources on seed germination and growth of tomato plants.

Materials and methods

Preparation of liquid seaweed extracts

Four algal species, two green, *Ulva lactuca* Linnaeus and *Caulerpa sertularioides* Gmelin, and two brown, *Padina gymnospora* (Kützing) Sonder and *Sargassum liebmannii* J. Agardh seaweeds, were collected from the intertidal zone at low tide, in May and November 2009, from the coastal area of Jalisco, Mexico, in Bahia Tenacatita (19° 28″N, 104° 84″W) and Bahia Careyitos (19° 43″N, 105° 02″W). Seaweed species were collected by hand and washed with seawater to remove debris, shells, and sand. Samples were transported to the laboratory in plastic bags, washed with tap water to remove surface salt, oven-dried for 72 h at 60 °C, and then ground in an electric mill (IKA-M 20) to less than 0.50 mm. This milled material (100 g) of each sample was subjected to acid digestion and analyzed by atomic absorption spectrophotometry for mineral analysis of sodium, potassium, calcium, and phosphorus (by colorimetry) following procedures from the Association of Official Analytical Chemists (AOAC 1990). Then, 100 g of each sample was added to 1 L of distilled water with constant stirring for 15 min followed by autoclaving at 121 °C for 1 h at 1.21 kg-cm^{-2}. The hot extracts were filtered through a Whatman No. 40 filter paper and stored. The liquid seaweed extracts were designated as liquid seaweed extracts (LSEs) stock solution and coded according to the genus and species: *U. lactuca* (UL), *C. sertularioides* (CS), *P. gymnospora* (PG), and *S. liebmannii* (SL). Furthermore, the pH and electrical conductivity (EC) of the LSEs were measured using a pH meter and conductivity meter, respectively, and the latter is expressed as dS m^{-1}. Finally, the color of the seaweed extracts was was observed visually. All determinations were performed in triplicate.

Bioassay for germination test

Experiments were conducted using certified tomato seeds (*S. lycopersicum* cv. Rio Fuego; Cal-Oro Vegetable Seeds, United Genetics, Inc., USA). Germination was observed daily over a period of 8 days according to methods of the Association of Official Seed Analysts (AOSA 2005). Four groups of 100 seeds were tested for germination per treatment (AOSA 2005). Experimental units were arranged in a randomized complete block design. Before treatment with LSEs, tomato seeds were surface-sterilized in 4 % sodium hypochlorite solution for 10 min and subsequently triple-rinsed in sterile distilled water prior to soaking in the seaweed extracts. Tested tomato seeds were placed on a Whatman No. 5 filter paper in sterilized 90-mm Petri dishes and then treated with 5 mL distilled water (control) or different concentrations of LSEs (0.2, 0.4, and 1.0 %). The plates were incubated at 25±1 °C and 16-h light/8-h dark regime. Germination was considered to have occurred once the radicle had protruded more than 2 mm.

Measured variables included germination percentage (GP), germination index (GI), mean germination time (MGT), and germination energy (GE), as well as seedling vigor index (SVI), plumule length, radicle length, total plant height, and dry weight of tomato seedlings. After 12 days, the LSE effects on seed germination and growth of tomato seedlings were measured. Plumule length, radicle length, and total plant height were measured with a vernier caliper. Dry weight was obtained with an electronic balance after oven-drying at 60 °C.

Parameters were calculated as follows: GP=(number of-germinated seeds/total number of seeds)×100. GI was calculated as described by the Association of Official Seed Analysts (AOSA 1983), following the equation: GI=$\sum$(Gt/Tt), where Gt is the number of seeds germinated on day t and Tt is the number of days. MGT was estimated according to Ellis and Roberts (1981) and expressed as days. MGT=$\sum(D\times n)/\sum n$,

where n is the number of seeds germinated on day D and D is the number of days counted from the beginning of the test. Seed GE was calculated according to the formula (GE %)=(numberofgerminatingseeds/numberoftotalseedsper-treatment after germination for 3 days)×100. SVI was determined according to Orchard (1977) by the following formula: SVI=(seedling length(cm)×germination percentage).

Greenhouse growth bioassay

Tomato plants were grown in a growth chamber under 16-h light regime at 25 °C and 8-h dark regime at 18 °C in sterilized soil peat moss (Sunshine Mix 3™). Two hundred fifty 15-day-old plants were selected and randomly assigned to different treatment groups and transplanted into pots containing a sand, perlite (Termolita S.A., Mexico), volcanic rock, and peat moss (Sunshine Mix 3™) (1:1:1:1 *w/w*) soil mix. Plants were fertilized 1 week after transplanting and treated with 50 mL of 20:20:20 (N–P–K) soil drench solution (Peters Professional; Scotts-Sierra Horticultural Products Co., USA). Thereafter, the plants were irrigated using the LSEs (50 mL every week). Plants were also irrigated separately with water (50 mL every third day). Potted plants were grown for 7 weeks in a greenhouse at ~25±2 °C, in 85 % relative humidity. Morphological characteristics such as shoot length, root length, total height, fresh weight, and dry weight were measured.

The experimental design and treatment were similar to those reported by Crouch and van Staden (1992). A total of 25 different treatments were used with ten replications. The first treatment served as the control in which plants were grown without LSEs. Three factors were randomized for the other 24 LSE treatments. The first factor was LSE applications method (foliar spray versus soil drench). The second factor was the type of seaweed species used to produce the LSEs. The third factor was the concentration (0.2, 0.4, and 1.0 %). The experimental units were arranged in a completely randomized tri-factorial design receiving either 50 mL distilled water for the control or 50 mL LSEs for the experimental treatments.

Statistical analysis

All data were analyzed for significant differences by analysis of variance (ANOVA) with mean separation using least significant difference (LSD). In the first experiment, one-way ANOVA was carried out for each parameter studied to assess significant differences. In the second experiment, three-way ANOVA assessed significant differences at the 5 % level. All statistical analyses were performed with Statistical Package STATGRAPHICS® Centurion XV for Windows.

Results

Macroelements in seaweeds and physicochemical content of LSEs

Results obtained from the nutrient analysis showed the presence of the macroelements Na, K, Ca, and P in all samples. The concentration of Na was higher in the green seaweeds *U. lactuca* and *C. sertularioides*, but K concentration was higher in the brown seaweeds *P. gymnospora* and *S. liebmannii*. The concentration of Ca was higher in *P. gymnospora* and *C. sertularioides*. The P concentration was low in all seaweeds (Table 1). The pH values for LSEs of *U. lactuca* and *P. gymnospora* were neutral and slightly acidic than those for LSEs made from *C. sertularioides* and *S. liebmannii*. The value of EC increases in all LSEs with an increase in the concentrations (Table 2).

Effect of LSEs on seed germination and growth of tomato seedlings under laboratory conditions

Germination occurred in all treatments after 2 days. The effect of the liquid seaweed extracts of *U. lactuca* and *P. gymnospora* at a concentration of 0.2 % gave a significant ($P \leq 0.05$) increase in GP over control after 2 days (Fig. 1a). Data indicated that the same treatments at concentrations of 0.4 and 1.0 % decreased the GP (Fig. 1b, c). Treatments of *C. sertularioides* and *S. liebmannii* had an inhibitory effect on seed germination. All concentrations delayed germination and GP dropped off with a higher concentration (1.0 %) (Fig. 1c).

Seeds treated with *U. lactuca* and *P. gymnospora* LSEs showed higher germination rate associated with lower MGT and greater seedling vigor (Table 3). LSEs of *U. lactuca* and *P. gymnospora* at 0.2 % showed high GP (75 and 76 %, respectively), elevated GI (9.8 and 10, respectively), a reduction in MGT (5.6 days), an increase in GE (88.7 and 87.2 %, respectively), and enhanced SVI (1,026.7 and 1,262.3, respectively). In contrast, seeds treated with LSEs of *C. sertularioides* and *S. liebmannii* at 1.0 % had the longest average delay (high

Table 1 The content of macroelements in seaweed species (g·100 g^{-1}, dry wt.)

Species	Na	K	Ca	P
U. lactuca	5.57±0.80	1.85±0.30	1.88±0.06	0.10±0.08
C. sertularioides	4.42±0.40	0.47±0.40	3.10±0.50	0.20±0.08
P. gymnospora	1.81±0.50	4.27±0.60	3.65±0.40	0.10±0.08
S. liebmannii	1.56±0.40	4.54±1.00	1.85±0.08	0.17±0.05

Values are average ± standard error (n=3)

Table 2 Physicochemical content of liquid seaweed extract (LSE) treatments of *U. lactuca* (UL), *C. sertularioides* (CS), *P. gymnospora* (PG), and *S. liebmannii* (SL)

Treatments (%)	Color	pH	EC (dS m^{-1})
Control		7.0	0
UL 0.2		7.00±0.50	0.99±0.11
UL 0.4	Greenish yellow	7.30±0.50	1.74±0.11
UL 1.0		7.36±0.50	3.57±0.11
CS 0.2		6.71±0.50	1.01±0.20
CS 0.4	Dark green	6.96±0.50	1.85±0.20
CS 1.0		6.99±0.50	3.93±0.20
PG 0.2		7.10±0.20	0.77±0.10
PG 0.4	Brownish red	7.60±0.20	1.43±0.10
PG 1.0		7.60±0.20	2.98±0.10
SL 0.2		6.19±0.50	1.01±0.15
SL 0.4	Brown	6.26±0.50	2.61±0.15
SL 1.0		6.37±0.50	3.99±0.15

Values are average ± standard error (n=3)

EC electrical conductivity

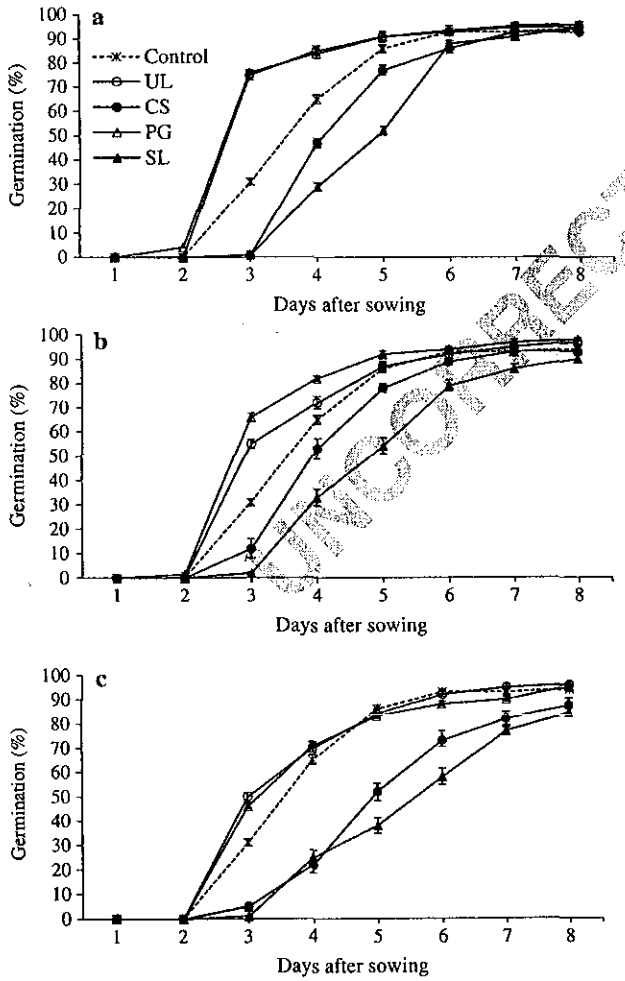

Fig. 1 Germination percentage of tomato seeds treated with liquid seaweed extracts at concentrations of **a** 0.2 %, **b** 0.4 %, and **c** 1.0 % from *U. lactuca* (*UL*), *C. sertularioides* (*CS*), *P. gymnospora* (*PG*), and *S. liebmannii* (*SL*). Values are presented as average (n=400 seeds); *bars* represent standard error

MGT), were the latest to germinate, and had the greatest spread of germination over time (Table 3).

The LSEs had a significant effect ($P \leq 0.05$) on growth of tomato seedlings. All LSEs showed a stimulatory effect on plumule length. The highest average plumule length was found in plants that received LSEs from *U. lactuca* and *P. gymnospora* at 1.0 % (7.3 and 8.3 cm, respectively; Fig. 2a). Moreover, these results indicate that LSEs of *U. lactuca*, *P. gymnospora*, and *S. liebmannii* promoted radicle length. The highest average radicle length was found in plants that received LSEs from *U. lactuca*, *P. gymnospora*, and *S. liebmannii* at 0.2 % (4.1, 5.8, and 5.5 cm, respectively) in comparison to the control. Furthermore, all *C. sertularioides* treatments had an inhibitory effect on radicle length (2.2 to 2.8 cm, Fig. 2b). All *C. sertularioides* and *S. liebmannii* LSE treatments significantly increase dry weight of tomato plants, as did *U. lactuca* and *P. gymnospora* LSEs at (1.0 %). The highest dry weight (0.036 g) was higher for the brown algae LSEs (Fig. 2c).

Effect of LSEs on tomato seedling growth in greenhouse

In the greenhouse experiment, both foliar spray and soil drench of LSEs showed a significant enhancement ($P \leq 0.05$) on growth of tomato plants. Application as a soil drench was found to be more effective in influencing the height of the plant (up to 79 cm) than the foliar spray application (75 cm).

The interaction between application form, treatment, and concentration demonstrates that plants treated with foliar sprays of *U. lactuca* at 1.0 % and *P. gymnospora* at 0.2 % displayed an increase in shoot length (47 and 49 cm), but plants receiving the same extracts as soil drench showed no significant difference in shoot length (Fig. 3a, b). Treatments applied as foliar spray of *U. lactuca* and *P. gymnospora* at 0.2 % displayed an increase in root length (25 and 26 cm, respectively) and total plant height (70 and 75 cm, respectively). Similarly, soil drench applications

t3.1 **Table 3** Effects of liquid seaweed extract (LSE) treatments on germination parameters of tomato seeds: germination percentage (GP), germination index (GI), mean germination time (MGT), germination energy (GE), and seedling vigor index (SVI)

t3.2 LSEs (%)	Parameters				
t3.3	GP	GI	MGT (days)	GE (%)	SVI
t3.4 Control	31±1.40 c	8.9±0.24 f	5.9±0.08 ef	68.8±2.76 d	763.4±40.8 b
t3.5 UL 0.2	75±2.00 g	9.8±0.89 hi	5.6±0.06 a	88.7±2.76 e	1,026.7±40.8 ef
t3.6 UL 0.4	55±1.85 e	9.4±0.86 gh	5.8±0.08 b	74.8±2.76 d	946.8±40.8 de
t3.7 UL 1.0	50±1.62 de	5.2±0.54 b	5.8±0.11 bc	72.9±2.76 d	1,096.5±40.8 fg
t3.8 CS 0.2	1±0.60 a	8.2±0.72 e	6.3±0.09 h	49.8±2.76 c	805.7±40.8 bc
t3.9 CS 0.4	12±3.93 b	9.0±0.93 fg	6.2±0.24 g	56.9±2.76 c	891.6±40.8 cd
t3.10 CS 1.0	5±0.91 a	3.4±0.75 a	6.5±0.10 i	24.5±2.76 a	622.9±40.8 a
t3.11 PG 0.2	76±1.10 g	10±0.67 i	5.6±0.06 a	87.2±2.76 e	1,262.3±40.8 h
t3.12 PG 0.4	66±1.67 f	9.9±0.57 hi	5.7±0.05 a	84.5±2.76 e	1,229.7±40.8 h
t3.13 PG 1.0	46±2.10 d	9.1±0.92 fg	5.8±0.09 bcd	74.2±2.76 d	888.7±40.8 cd
t3.14 SL 0.2	1±0.40 a	7.9±0.85 de	5.9±0.13 def	31.3±2.76 ab	1,160.6±40.8 gh
t3.15 SL 0.4	2±0.57 a	7.5±1.02 d	5.9±0.16 cde	36.0±2.76 b	873.2±40.8 bcd
t3.16 SL 1.0	1±0.31 a	6.6±1.13 c	6.0±0.18 f	28.5±2.76 ab	635±40.8 a

Values are average ± standard error (n=400). Average followed by the same letter within columns is not significantly different, according to LSD multiple range test ($P \leq 0.05$)

UL U. lactuca, CS C. sertularioides, PG P. gymnospora, SL S. liebmannii

of *U. lactuca* at 1.0 % and *P. gymnospora* at 0.2 % resulted in an increase in root length (33 and 32 cm, respectively) and total plant length (79 cm) (Fig. 3c–f).

In addition, positive effects on fresh weight were observed with application as foliar spray of *P. gymnospora* at all concentrations (9.9, 10.1, and 10.7 cm) and with application as soil drench of *U. lactuca* and *C. sertularioides* at 1.0 % (14 and 14.6 cm, respectively) (Fig. 4a, b). Dry weight of tomato plants was unaffected by treatment with LSEs, except for *S. liebmannii* that produced negative effects (Fig. 4c, d).

Discussion

Previous papers reported that seaweeds contain high macroelement levels (Ca, K, P), especially those from the Phaeophyta (Hong et al. 2007). The content of minerals in the seaweeds used in this research was in general agreement with the typical values for these marine algae from other countries (Gireesh et al. 2011; Hong et al. 2007; Kalaivanan and Venkatesalu 2012; Sivasangari et al. 2010) and Mexico (Carrillo-Domínguez et al. 2002; Robledo and Freile Pelegrin 1997). The quantitative ranges for the various components in seaweed can vary due to season, location, and analytical methods (Castro-González et al. 1996; Ito and Tsuchiya 1981).

The pH and EC from LSEs affected germination in tomato. The beneficial effects of seaweed extracts may arise from higher seed moisture after the drying phase (Weges and Karsssen 1990). Additionally, changes in pH and EC of acidic and neutral extracts can affect bioactivity (Booth 1969; Henry 2005). According to Reinhardt and Rost (1995), most plants are more sensitive to salinity during germination and seedling growth. This is in agreement with our study whereby a higher germination percentage was found with treatments of *U. lactuca* and *P. gymnospora* (0.99 and 0.77 dS m^{-1} with 0.2 %) at low concentration. This may be due to the absence of salts in the medium, thereby allowing seeds to more efficiently imbibe water. In contrast, LSEs of *C. sertularioides* and *S. liebmannii* (3.9 dSm^{-1} with 1.0 %) at higher concentration showed a significant negative effect on the germination of tomato seeds, by inhibiting the seeds' ability to imbibe water.

Similar results have been reported by Basher et al. (2012). They evaluated the effect of seaweed, salt water, and drainage water on germination percentage, rate, and seedling growth of tomato (*Lycopersicum* spp.). The combination of water with seaweed treatments shows the highest rate for 2.7 dSm^{-1} with 0.1 % seaweed concentration and lowest rate for 5 dSm^{-1} with 0.05 % seaweed concentration compared to control. Salt stress reduced the growth of the plumule and radicle in tomato seedlings, and there was a direct adverse relation between NaCl concentration and reduction in growth (plumule and radicle) (Nyagah and Musyimi 2009). Tomato seedlings are moderate sensitive to NaCl salinity. However, during imbibition, the effect of salt is merely osmotic until a hydration threshold is surpassed (Almodares et al. 2007). Tomato seeds treated with low concentrations of *U. lactuca* and *P. gymnospora* responded better in terms of germination rate associated with lower MGT, high GI and GE, and consequently greater seedling vigor and

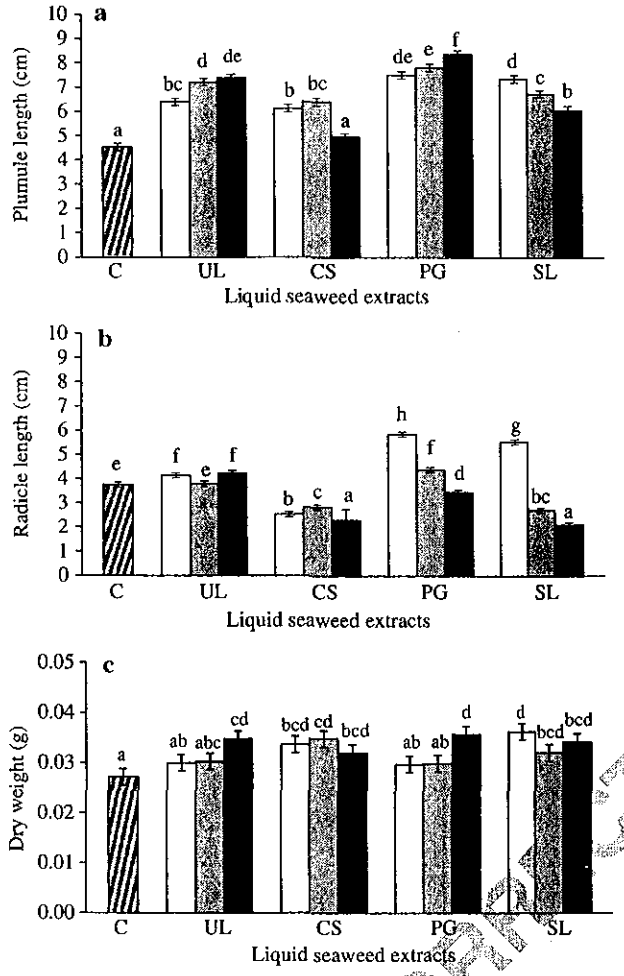

Fig. 2 Effect of liquid seaweed extract treatment applications on a plumule length, b radicle length, and c dry weight of tomato seedlings at different concentrations: 0.2 % (*white columns*), 0.4 % (*gray columns*), and 1.0 % (*black columns*). Treatments: control (*C*), *U. lactuca* (*UL*), *C. sertularioides* (*CS*), *P. gymnospora* (*PG*), and *S. liebmannii* (*SL*). Columns denoted by a *different letter* are significantly different at $P \leq 0.05$. Values represent average (n=300 seedlings); *bars* represent standard error

higher plumule and radicle length. The higher concentration showed a decreasing trend, particularly with *C. sertularioides* and *S. liebmannii* treatment at 1.0 %.

In tomato, high concentrations of salt in the germination media significantly delayed the onset, reduced the rate of germination (Foolad and Lin 1997, 1998), and reduced the germination percentage (Hajer et al. 2006). In this study, the reduction in germination and growth (plumule length and radicle length) following applications of LSEs of *C. sertularioides* and *S. liebmannii* could be a result of high-salinity extracts. Seed germination of tomato lines (LA3770, R205, CT6, FLA, and ME) was delayed by salinity increasing from 2.5 to 10 dS m^{-1} (Kaveh et al. 2011). In addition, germination rate decreased in tomato cultivars (Pascal, Red Stone, Shohba, Super Marmand, and Tanshet Star) at high salt concentration in proportion to the control application (Al-Harbi et al. 2008).

Salt stress decreased germination by preventing the germination processes due to the accumulation of a high concentration of Na^+ and Cl^- ions, which might be toxic to the embryo or the developing seedlings (Almodares et al. 2007), and can affect many vegetable crops, leading to uneven stand establishment and reduction of crop yields (Yildirim and Guvenc 2006). The mechanisms for salt damage during germination are not completely understood (Almodares et al. 2007). However, the effect of salinity on plant growth is a complex syndrome that involves osmotic stress, ion toxicity, and mineral deficiencies (Musyimi et al. 2007).

In the present study, higher concentrations of seaweed extract from *C. sertularioides*, *P. gymnospora*, and *S. liebmannii* at 1.0 % had toxic effects on tomato seedlings and caused detrimental effects such as radicle browning and disintegration of plumules. This detrimental effect for tomato growth has been reported previously by Arnon and Johnson (1942) and is caused by higher pH in the growth medium. Higher concentrations of seaweed extracts from *S. johnstonii* (2.0 to 10 %) caused the same detrimental effects in black gram (*Vigna mungo*) seedlings (Kumari et al. 2011). Indeed, several studies have examined seaweed extracts on seed germination of various species such as table beet (Wilczek and Ng 1982), lettuce (Moller and Smith 1998), tomato (Demir et al. 2006), green gram (Ashok-Kumar et al. 2012), and black gram (Kalaivanan and Venkatesalu 2012; Ganapathy Selvam et al. 2013). The increased germination percentage at low concentrations could be due to the presence of growth-promoting substances such as indole acetic acid, indole-3-butyric acid, gibberellins, cytokinins, micronutrients, vitamins, and amino acids (Challen and Hemingway 1965).

Seaweed products exhibit growth-stimulating activities, and the use of seaweed formulations as biostimulants in crop production is well established. Seaweed ingredients include macro- and microelement nutrients, amino acids, vitamins, cytokinins, auxins, and abscisic acid that affect cellular metabolism in treated plants, leading to enhanced growth and crop yield (Crouch and van Staden 1993; Stirk et al. 2004; Wightman and Thimann 1980). In addition, seaweeds contain precursors of elicitor compounds that promote germination (Stevenson 1974), growth, and maintenance of plant health (Kloareg et al. 1996). Another possibility is the presence of polysaccharides in LSEs, as sugars are known to improve plant growth in a similar way to hormones (Rolland et al. 2002). Zeatin is another candidate for induction of rooting in plants by seaweed (Finnie and van Staden 1985).

Furthermore, brown and green seaweed extracts contain various betaines and betaine-like compounds (Blunden et al. 1986; Ghoul et al. 1995). In plants, betaines serve as a compatible solute that alleviates osmotic stress induced by salinity and drought stress. However, other roles have also been

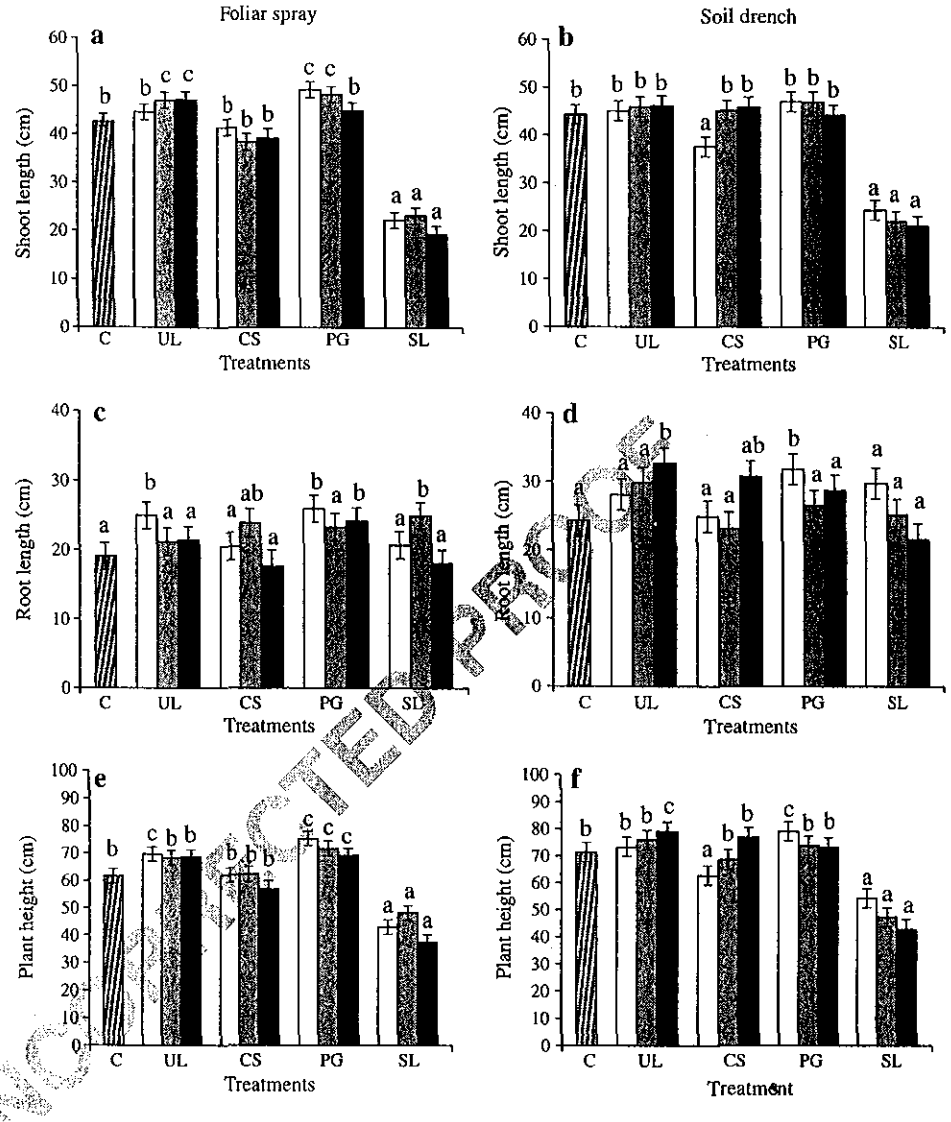

Fig. 3 Effect of liquid seaweed extract treatments applied as foliar spray and soil drench on **a**, **b** shoot length, **c**, **d** root length, and **e**, **f** total plant height of tomato at different concentrations: 0.2 % (*white columns*), 0.4 % (*gray columns*), and 1.0 % (*black columns*). Treatments: control (*C*), *U. lactuca* (*UL*), *C. sertularioides* (*CS*), *P. gymnospora* (*PG*), and *S. liebmannii* (*SL*). Columns denoted by a *different letter* are significantly different at $P \leq 0.05$. Values represent average ($n=10$ plants); *bars* represent standard error

suggested (Blunden and Gordon 1986). It has been indicated that betaine may work as a nitrogen source when provided in low concentration and serve as an osmolyte at higher concentrations (Naidu et al. 1987). Betaines have been shown to play a part in successful formation of somatic embryos from cotyledonary tissues and mature seeds of tea (Akula and Bateson 2000). All that information supports our results about seed germination and developed seedlings with LSEs.

Our results relate with those in the literature (Ashok-Kumar et al. 2012; Ganapathy Selvam et al. 2013; Kalaivanan and Venkatesalu 2012; Kumari et al. 2011; Sridhar and Rengasamy 2010, 2011), where seaweed extract at low concentration was used for a variety of plants without any harmful effects. Effect of seaweed extract on tomato seedlings was successfully demonstrated by Crouch and van Staden (1992, 1993). Tomato plants treated with LSE from *Ecklonia maxima* as a soil drench at 1.0 % increased root length and fresh weight, whereas foliar applications of the same product had no significant effect on shoot growth but showed effect on fresh weight at a concentration of 0.4 %. Also, seaweed extracts enhance nutrient uptake by roots (Crouch et al. 1990), resulting in root systems with improved water and nutrient uptake efficiency, thereby causing enhanced general plant growth and vigor. Our results agree with these trends. We showed that applications of LSEs from *U. lactuca* and *P. gymnospora* as a soil drench and foliar spray increased shoot length and root length of tomato plants. In another study, Kumari et al. (2011) reported an increase in root length, shoot length, and fresh weight in tomato treated with drench and foliar applications of LSE from *S. johnstonii*. Enhanced vegetative growth in tomato plants treated with a higher concentration of seaweed extract could be due to the mineral nutrients

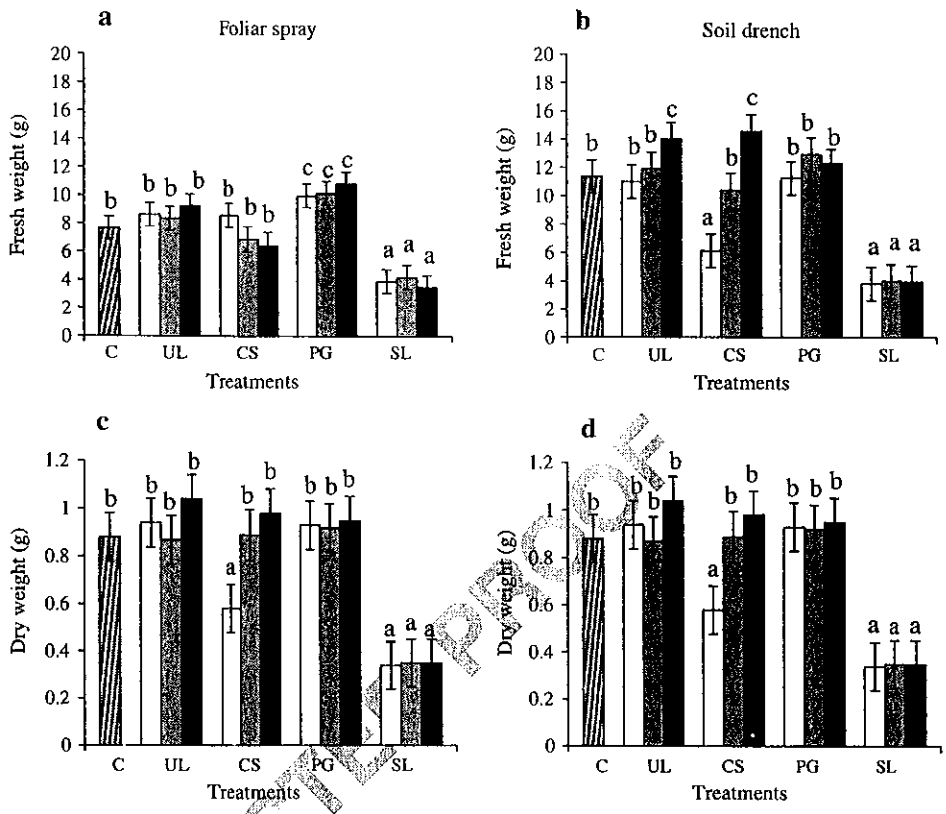

Fig. 4 Effect of liquid seaweed extracts treatments applied as foliar spray and soil drench on **a**, **b** fresh weight and **c**, **d** dry weight of tomato plants. Treatments: control (*C*), *U. lactuca* (*UL*), *C. sertularioides* (*CS*), *P. gymnospora* (*PG*), and *S. liebmannii* (*SL*). Columns denoted by a *different letter* are significantly different at $P \leq 0.05$. Values represent average ($n=10$ plants); *bars* represent standard error

present in the LSE. The beneficial effect of seaweed extract application can be attributed to its many components working synergistically at different concentrations (Fornes et al. 2002). Similar, studies conducted with LSEs applied by sprays under controlled experiments resulted in increased height and improved root growth in tomatoes (Verkleij 1992; Zodape et al. 2011). Other positive results were reported in spinach and tomatoes (Featonby-Smith and van Staden 1983; Finnie and van Staden 1985). This enhanced growth effect is thought to be due to various organic compounds present in the seaweed extracts. In the present research, it was not possible completely establish the relationship between minerals and growth of tomato plants. However, a possible explanation for these results may be due to the presence of P in LSEs. The LSEs or liquid seaweed fertilizers can help stimulate root proliferation and enhance root-to-shoot ratio, thereby making the plants more able to mine adequate nutrients from the deeper soil layers and influence crop maturity as a whole. Since liquid seaweed fertilizer is a very good source of K, it helps in regulating the water status of the plants, controls the opening and closing of stomata, and thereby, to a large extent, controls the photosynthesis. Meristematic growth, translocation of photosynthates, and disease resistance are also influenced by the presence of K. The Ca in seaweed extracts helps in enzyme activation, cell elongation, and cell stability. The organic constituents of seaweed extract include plant hormones which elicit strong physiological responses in low doses (Pramanick et al. 2013).

In conclusion, this study shows that liquid seaweed extracts from *U. lactuca* and *P. gymnospora* were more effective at stimulating the growth of tomato seedlings, and therefore, they are potential candidates for the production of effective biostimulants. Surprisingly, the extracts of both species showed better results when they were applied at lower concentrations than more concentrated extracts. This shows that only a small amount of seaweed extract can be used or even could be mixed with commercially available fertilizers to enhance plant growth. In places where inorganic fertilizers are limited, LSEs may provide a powerful and environmentally friendly approach to nutrient management. This study provides valuable information on the identification and utilization of Mexican seaweed resources for agriculture. The presence of inorganic minerals in LSEs makes them an excellent choice as organic fertilizers. The practice of applying eco-friendly seaweed extract can therefore be recommended to growers to help attain better germination and growth of tomato or other crops. Future studies on specific mechanisms attributed to plant growth are required to determine the potential of *U. lactuca* and *P. gymnospora*

as commercial growth biostimulants which can be promoted as eco-friendly biofertilizers across Mexico.

Acknowledgments The authors are grateful to Prof. Dr. Carla Vanessa Sánchez Hernández from the "Departamento de Producción Agrícola, Centro Universitario de Ciencias Biológicas y Agropecuarias (CUCBA) Universidad de Guadalajara" for her valuable suggestions during this research. Gustavo Hernández wishes to express his thanks for the fellowship granted under the program "Beca de exclusividad" of the "Comisión de Operación y Fomento de Actividades Académicas del IPN (COFAA)" and also the program "Estímulo al Desempeño de los Investigadores (EDI) del IPN."

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VI. EXTRACTOS ETANÓLICOS DE ALGAS MARINAS COMO INDUCTORES DE DEFENSA

Induced protection against early blight in tomato (*Solanum lycopersicum* L.) is elicited by seaweed extracts.

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Abstract - A number of reports have shown that crude or purified algal preparations provide protection against pathogens in plants. In this work, the protective effect against infection by *Alternaria solani*, conferred in tomato plants by the application of polysaccharide-rich algal cell wall preparations was tested. These were obtained from four species of temperate and warm-water algae, namely two green, *Ulva lactuca* (Linnaeus) and *Caulerpa sertularioides* (Gmelin), and two brown, *Padina gymnospora* (Kützing) Sonder and *Sargassum liebmannii* (Agardh), algae. Apart from *U. lactuca*, little information is available regarding the other three temperate/tropical algae. All extracts were shown to confer resistance to *A. solani*, particularly those obtained from *U. lactuca* and *P. gymnospora*. It was evident from the protein activity and gene expression assays that *U. lactuca* induced resistance against this necrotrophic fungus through the expression of systemic wound response (SWRP) genes, including defense, signal pathway and protease genes. Increasingly weaker correlations between SWRP protein activity and gene expression and resistance against *A. solani* were observed for *C. sertularioides*, *P. gymnospora* and *S. liebmannii*. This suggests that defense mechanisms other than the jasmonic acid/systemin- wound response pathway are induced by cell wall polysaccharide preparations of the latter three algae.

Keywords

Seaweed extracts; induced resistance; defense-related proteins; plant defense; *Alternaria solani*; systemic wound responsive genes

Introduction

Plants induce a complex array of defense response against microbial pathogens that include a transient oxidative burst, changes in intracellular calcium concentration, activation of signaling pathways depending on jasmonic acid (JA), ethylene (ET) and salicylic acid (SA), synthesis of phytoalexins, changes in cell wall composition, and induction of pathogenesis-related (PR) proteins, among others (Kombrink and Somssich 1995; Kuc 2006). Defense responses occur after perception of signal molecules called elicitors derived from pathogens (e.g. pathogen associated molecular patterns, PAMPs) or from the host plant (e.g. pectin fragments, cellodextrins) (Ebel and Cosio 1994; Cotê et al. 1998; Aziz et al. 2007). A wide variety of elicitors have been characterized, including oligo- and polysaccharides, peptides, proteins and lipids (Boller 1995; Creelman and Mullet 1997; Cotê et al. 1998; Klarzynski

et al. 2000). Polysaccharides obtained from green, brown and red marine macroalgae can also act as elicitors of plant defense responses and enhanced resistance against viral, fungal and bacterial pathogens (Klarzynski et al. 2003; Aziz et al. 2003; Cluzet et al. 2004; Laporte et al. 2007; Trouvelot et al. 2008; Pauler et al. 2009; Jaulneau et al. 2011; Vera et al. 2011a), nematodes (Crouch and van Staden 1993) and herbivores (Jaulneau et al. 2010; Sangha et al. 2011). Numerous algal elicitors have been identified such as linear β-1,3 glucans (laminarin), β-1,3 sulphated fucans, carrageenans and ulvans (Mercier et al. 2001; Klarzynski et al. 2000, 2003; Ménard et al. 2004; Jaulneau et al. 2010). The structure and composition of algal oligosaccharides are critical for the activation of signaling pathways regulating defense responses. For example, in tobacco, sulphated laminarins induced SA levels, expression of ET- and SA-dependent PR proteins and protection against tobacco mosaic virus, while laminarin only induced expression of ET-dependent PR proteins (Ménard et al. 2004).

A number of reports have shown that crude or purified algal preparations provide protection against pathogens in plants (Cluzet et al. 2004; Paulert et al. 2009, 2010). For instance, *Ulva armoricana* extracts application reduced foliar disease incidence of three powdery mildew pathogens on common bean, grapevine and cucumber (Jaulneau et al. 2011). Similar results were obtained after applications of seaweed extracts from *Ascophyllum nodosum* to enhance foliar resistance to *Phytophthora capsici* in pepper (Lizzi et al. 1998), *Alternaria radicina* and *Botrytis cinerea* foliar blights in carrots (Jayaraj et al. 2008), and *A. cucumerinum*, *Didymella applanata*, *Fusarium oxysporum*, and *B. cinerea* in cucumber (Jarayaj et al. 2011).

Due to their effects as plant protectants, algal extractions represent an alternative tool for disease control in crops. Marine macroalgae are abundantly available in Mexican temperate and tropical waters, and represent a naturally occurring source of molecules that can be used to enhance resistance for controlling plant disease. Thus, the purpose of this study was to evaluate the biological effects of seaweed extracts obtained from brown and green macroalgae against early blight (*Alternaria solani*) in tomato plants. With the exception of *U. lactuca*, not much information is available regarding the pathogen defense-inducing capacity of the other three algal species included in this study, which in contrast to cold water algae, have been only marginally studied. The potential to induce resistance was evaluated by measuring the activity and expression of key defense-related proteins and genes.

Materials and methods

Algae material and extract preparation

Four seaweed species, representing two green *Ulva lactuca* (Linnaeus), *Caulerpa sertularioides* (Gmelin), and two brown *Padina gymnospora* (Kützing) Sonder and *Sargassum liebmannii* (Agardh) algae were collected from the surface of rocks at low tide, from the coastal area of West Mexico (in the state of Jalisco), in Bahía Tenacatita (19° 28'N, 104° 84'W) and Bahía Careyitos (19° 43'N, 105° 02'W) in May and November 2009. Seaweeds species were collected by hand, washed with seawater to remove debris, shells and sand. Samples were transported to the laboratory in plastic bags, washed with tap water to remove salt from the surface, oven-dried for 72 h at 60°C, and ground into a fine powder.

The extraction protocol was designed to obtain water-soluble cell wall components and was similar to that reported by Cluzet et al. (2004). Dried algal powder (100 g) was extracted in 1 L distilled water, autoclaved at 110°C for 2 h and then filtered through filter paper. The filtrate was concentrated to 200 mL under vacuum with a Büchi R-215 rotary evaporator (BUCHI Labortechnik AG, Switzerland). The soluble compounds were precipitated with 500 mL of ethanol for a period of 48 h at –20°C. The precipitate was recovered by filtration and lyophilized. The dried fraction was weighed and dissolved in distilled water. Extracts were labeled as follows: *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Padina gymnospora* (PG) and *Sargassum liebmannii* (SL).

Plant material and treatments

Tomato plants (*Solanum lycopersicum* cv. Rio Fuego; Cal-Oro Vegetable Seeds, United Genetics, Inc., Gilroy, CA, USA), were grown in pots using a rich soil mixture, watered daily and fertilized weekly with a 20–20–20 (N–P–K) soil drench solution (Peters Professional; Scotts-Sierra Horticultural Products, Marysville, OH, USA). The plants were maintained in a bioclimatic chamber maintained under controlled conditions of light, temperature and photoperiod (16 h light at 28°C/ 8 h dark at 16°C).

After 30 days, tomato plants were foliar sprayed with seaweed extract solutions (UL, CS, PG and SL, at 0.1 mg mL^{-1}) or water (controls) until run-off. Tween-20 (Sigma-Aldrich Inc., St Louis, MO, USA) was added to a final concentration of 0.01 % to improve absorption of the sprayed solution. For protein assays, leaf samples were collected at 0, 24, 48 and 72 h, after treatment and immediately frozen in liquid

nitrogen and stored at –80ºC. The experiments were performed independently twice (n = 8 plants for each treatment). Samples destined for qPCR (n = 3 plants for each treatment) were sampled 24 h after treatment.

Protection assays

A pure culture of the fungal pathogen *Alternaria solani* was isolated from infected tomato tissue and maintained on PDA media for 30 days at 25°C. Eight tomato plants were infected with the fungus 24 h after algal treatments (UL, CS, PG and SL, at 0.1 mg mL^{-1}) or water controls. For this, a conidial suspension (1 × 10^6 conidia mL^{-1}) of *A. solani* was inoculated on a leaflet of six different leaves. Plants were then transferred to a greenhouse and grown under a 100% humidity atmosphere. Disease severity was evaluated 15 days after inoculation (dai) using a six-point disease rating scale, based on the percentage of leaf area infected as described by Jayaraj and Punja (2007): 1 = 0%; 2 = 1-10%; 3 = 11-25%; 4 = 26-40%; 5 = 41-55%; 6 = >56%. Percent disease index was calculated as the sum of disease ratings of individual leaves/ total number of leaves × 100/ maximum rating (Jayaraj et al. 2008). The experiment was conducted twice.

Protein Activity Assays

Plant tissue (30mg) was grounded in liquid nitrogen and homogenized in 400 µl doubly distilled and de-ionized water. The homogenate was centrifuged at 14 000 rpm for 20 min at 4°C and the supernatant was used directly for protein content and activity. The plant extracts were always assayed immediately following preparation. Inhibition of bovine trypsin activity was assayed according to Erlanger et al. (1961), using Na-benzoyl-L-arginine-p-nitroanilide hypochloride (BApNA) as substrate. Activity was expressed as units of inhibitory activity per mg of protein content. Polyphenol oxidase (PPO) and peroxidase (POD) activities were measured according to Thaler et al. (1996). For PPO, plant extracts were added to a caffeic acid solution (2.92 mM in pH 8 potassium phosphate buffer); for POD the procedure was identical, except that a guaiacol solution, prepared in the same buffer, was used instead. Both activities were estimated by an increase in absorbance at 470 nm, determined after 3 min, and calculated per mg total protein. Protein content was measured according to the Bradford method (Bradford 1976), employing a commercial kit (Bio-Rad Laboratories, Hercules, CA, USA). All enzymes and substrates employed

were from Sigma-Aldrich. Each protein activity assay was determined in protein extracts prepared independently from 8 plants.

Gene Expression Analysis by qPCR

Leaf RNA isolation was performed using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Isolated RNA was treated with DNAase and purified with the RNeasy kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocols. After RNA extraction, 4 µg was reverse transcribed with M-MLV reverse transcriptase (Promega, Madison, WI, USA) by using oligo-$dT_{(12-18)}$ primers. Real-time PCR amplifications were performed using SYBR Green detection chemistry in a CFX96 Touch apparatus (Bio-Rad). Reactions were prepared in a total volume of 20 µl containing: 2µl of first strand cDNA template, 2 µl each of forward and reverse primers (2 µM), 8 µl of iQ™ SYBR® Green Supermix (Bio-Rad) and 6 µl of sterile de-ionized-distilled water. Gene-specific primers for defense-related genes, allene oxide synthase (*AOS*), lipoxygenase (*LOXD*), MYC transcription factor (*JAMYC2*), Phospholipase A2 (*PLA2*), wound-inducible carboxypeptidase (*SCP*), phenylalanine ammonia lyase (*PAL*), cysteine proteinase (*CYP2*), wound-induced proteinase inhibitor I (*PI-I*) and wound-induced proteinase inhibitor II (*PI-II*) were designed based on GenBank or Sol genomics network reported sequences (Table 1). Elongation factor 1-α (*EFα1*) and a *TIP41*-like family gene (*TIP41*) (Expósito-Rodríguez et al. 2008) were employed as endogenous control genes. The cycling conditions were set as follows: initial denaturation step at 95°C for 5 min to activate the iTaq™ DNA Polymerase, followed by 40 cycles of denaturation at 95°C for 15 sec and annealing at 60°C for 1 min. At the end of PCR amplification, a melting curve analysis was immediately performed to confirm the specificity of the reactions. Baseline and threshold cycles (Ct) were automatically determined using Real-Time PCR System software. Efficiencies for all genes tested were greater than 95%. Relative expression was calculated using the comparative cycle threshold method (Livak and Schmittgen 2001), where delta (Δ) cycle threshold of cDNA from controls was defined as 100% transcript presence. Transcript abundance data were normalized against the average transcript abundance of two endogenous control genes, as determined during the assays. The fold change in expression of the target genes in each treatment was calculated using the following equation: $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct = (Ct\ target\ gene - average\ Ct\ reference\ genes)_{treatment} - (Ct\ target\ gene - average\ Ct\ reference\ genes)_{control}$.

Statistical analysis

Data in al assays were analyzed by One-way ANOVA followed by Fisher's least significant difference (LSD) procedure using the STATGRAPHICS program, version Centurion XV.II for Microsoft windows (Stat Point, Inc.).

Results

Algal extract treatments induce protection against A. solani in tomato

Tomato plants treated with algal extracts and then inoculated with *A. solani* consistently showed reduced disease development. Thus, plants treated with seaweed extracts (UL, CS, SL and PG) developed significantly less infected leaf area than control plants. The total surface of infected leaves, measured as percent disease index was reduced by 65%, 61%, 58% and 64%, respectively (Fig. 1). In this test, UL and PG treatments induced the highest protection against *A. solani* infection. Similar results were obtained in additional independent experiments. Higher concentrations of the algal extract solutions (0.5, 1.0, 5.0 and 10 mg mL^{-1}) showed evident signs of plant injury, such as necrotic spots, leaf burn and rolling at the margins (data not shown).

Effect of algal treatment on activity of defense-related proteins

A significant increase in the activity of defense-related proteins, including polyphenol oxidases (PPO) peroxidases (POD) and proteinase inhibitors (PIs) was observed in tomato plants after spraying with seaweed extracts, as compared to water controls (Fig. 2). A sharp increase in PPO activity (3-fold increase) was seen at 72 h after UL treatment. A minor significant increase was also observed after SL and PG (48 h) treatments (Fig. 2a). Maximum levels of POD activity were observed at 72 h after CS treatment (2-fold increase). However POD activity increased more dramatically in PG-treated plants, particularly at 48 and 72 h, where a 4-fold increase was observed (Fig. 2b). In contrast to PPO and POD activity, induction of PI levels was pronounced after all four seaweed treatments. This was evident in UL and SL-treated plants, where accumulation was observed after 24 h and maintained until 72 h. Similar to PPO activity UL-treated plants also presented the maximum PI levels, with a three-

fold increase detected at all times tested. PG-treated plants also induced a significant accumulation after 48 h, while CS treatment showed increase only at the later interval. In the latter cases, accumulation was less than two fold (Fig. 2c).

Effect of algal treatment on gene expression

A differential response to seaweed treatment was observed after measuring SWRP gene expression (Fig. 3). Treatment of tomato plants with UL extracts induced defense, signal pathway and protease genes. *PLA2* and *CYP* transcripts accumulated to higher levels, reaching almost a twelve and ten-fold increase, respectively. In contrast only *PAL* expression was down regulated. Even though CS and SL-treated plants showed almost no induction of SWRP genes, SL extracts increased *PAL* expression. The same treatment also repressed signal pathway genes expression (*LOX*, *AOS* and *JAMYC*). PG-treated plants had no effect on signaling gene expression however *CYP* and both wound-induced proteinase inhibitors (*PI-I* and *PI-II*) were up-regulated with an approximately two and three-fold increase, respectively.

Discussion

Seaweed extracts stimulated defense against the necrotrophic pathogen *Alternaria solani*. In this study, we report the direct involvement of PPO, POD and PI induction during the treatment of tomato plants with seaweed extracts at 0.1 mg mL^{-1} compared with the controls. The maximum induction of the plant defense responses (both in terms of gene expression and protein activity), observed in UL and PG, coincided with fewer necrotic lesions and a larger disease reduction. This was indicative that UL and PG extracts were the most active elicitors of plant defense against *A. solani*. It appears that UL might exert this protective effect through the induction of the JA and systemin dependent systemic wound responsive gene expression and protein activity. This is in agreement with the results of Jaulneau et al. (2010) which showed that ulvan, a sulfated polysaccharide present in *Ulva spp.* extracts, activated plant immunity in *Medicago truncatula* and Arabidopsis through the JA signaling pathway. It is also consistent with results obtained with tomato plants impaired in the JA signaling pathway mediated by the systemin polypeptide, which became more susceptible to infection by the fungal

necrotroph *B. cinerea* (Díaz et al. 2002; El Oirdi et al. 2011). Similar results were obtained with extracts obtained from the marine brown macroalga, *Ascophyllum nodosum* which induced resistance in Arabidopsis to different pathogens, including the necrotroph *Sclerotinia sclerotiorum* via a predominantly JA dependent pathway (Subramanian et al. 2011). Conversely, the pattern observed in PG was weakly similar to that produced by UL, which suggests that other JA-responsive proteins/genes not tested in this work, perhaps also involving the participation of ET and perhaps SA (Vera et al. 2011a, b, 2012; Menard et al. 2004), might have been active against this pathogen. The same argument could be used to explain the results produced by the other two other algal extracts (CS and SL) which generated a weaker defense response, and showed almost no induction of SWRP genes and proteins. Such differences were to be expected considering the likeliness that the cell wall polysaccharide extracts utilized in this study differed widely in chemical composition, probably consisting of ulvans or ulvan-like polysaccharides in the green macroalgae UL and CS (Jaulneau et al. 2010) and laminarans, fucans and/or related polysaccharides (Klarzynski et al. 2003; Craigie 2011) in the brown macroalgae SL and PG. In this respect, differential responses to algal polysaccharides obtained from green and brown algal extracts were recently reported in tobacco (Laporte et al. 2007), whereas an emphatic example of the effect that even small differences in composition have in the elicitation of pathogen resistance was reported in Arabidopsis plants treated with iota- and lambda-carrageenans extracted from red algae (Sangha et al. 2010). Here, it was shown that pretreatment with the highly sulfated lambda-carrageenan induced resistance to *S. sclerotiorum*, mediated mostly by JA, whereas the less sulfated iota-carrageenan enhanced susceptibility. Latter data revealed that resistance against herbivory by *Trichoplusia ni* larvae in Arabidopsis, induced by the application of iota-carrageenan, was found to require a dual JA and SA response (Sangha et al. 2011). The results are also consistent with a recent study that revealed a functional relationship among JA, ET and SA signaling pathways in tomato defense against the similar necrotrophic fungal pathogen *Alternaria alternata* f. sp. *lycopersici* (Jia et al. 2013). In addition, resistance to fungal pathogens in cucumber and carrot, including *Alternaria*, was elicited by algal extracts obtained from the brown macroalgae *A. nodosum* (Jayaraj et al. 2008, 2011). In these cases, resistance was attributed to enhanced activity and expression of various defense-related enzymes including chitinase, β-1, 3-

glucanase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase (PAL), and LOX, and/or the accumulation of phenolic compounds. Laminaran-containing *A. nodosum* preparations have also been shown to up-regulate PAL, caffeic acid O-methyl transferase, LOX and SA as defense responses in tobacco (Patier et al. 1993; Potin et al. 1999; Klarzynski et al. 2000), and of antifungal compounds in alfalfa (Kobayashi et al. 1993).

Acquired resistance induced by algal extracts has been found to be proportional to the concentration of the extract and the number of applications (Lizzi et al. 1998). Compared to other reported studies, the algal cell wall polysaccharide extracts employed in this study were found to be within the range of concentrations previously reported to exert biological activity against pathogens. They also compared favorably with the antimicrobial activity of several plant extracts used to control *Alternaria solani in vitro* and *in vivo*, which at 5% concentration caused the highest reduction of mycelial growth of *A. solani* (Nashwa and Abo-Elyousr 2012). Moreover, the 0.1% concentration used to control *A. solani* in tomato was lower than reported applications of Stimplex™, a commercial formulation of extract seaweed derived from *A. nodosum*, in carrot and cucumber plants. Thus, a 0.2% concentration was applied to carrot plants to lower infection levels of *A. radicina* and *B. cinerea* 10 or 25 days after inoculation (Jayaraj et al. 2008), whereas the same treatments, but at 0.5% and 1% concentration, were used to control infection by *A. cucumerinum*, *B. cinerea, Didymella applanata* and *Fusarium oxysporum* in cucumber. However, the concentrations that were used are still high in comparison with microbial elicitors (e.g. 30 $\mu g\ mL^{-1}$) used in other studies (Keller et al. 1996; Villalba Mateos et al. 1997; Mercier et al. 2001).

Similar to our study, the treatments of carrot and cucumber plants were accompanied by higher activities of defense-related enzymes including POD and PPO, amongst others (Jayaraj et al. 2011). Also, the increased activities were stable and prolonged for up to 72 h.

Induction of these and other defense related proteins, including glutathione-S-transferase and proteinase inhibitors, has also been also reported as a result of foliar application of diverse algal extracts at concentrations ranging from 0.1 to 1 $mg\ mL^{-1}$ (Klarzynski et al. 2000, 2003; Azizi et al. 2003; Ménard et al. 2004, Cluzet et al. 2004; Laporte et al. 2007; Jaulneau et al. 2010).

In our study, it appears likely that extracts from seaweeds contain elicitors (carbohydrates) that induce defense in tomato plants. Our extraction protocol retained soluble cell wall components of seaweed which

are the best characterized algal elicitor material from green and brown marine macroalgae. It is proposed that the main compounds are ulvans, alginates, fucans, and laminaran (a β-1,3 glucan), all of which are known to trigger defense responses. The results obtained were in agreement with the general concept that marine algae represent an abundant and inexpensive source of bioactive compounds which can be used in agriculture (Craigie 2011). This was corroborated in this study, in which we identified specific biological activity on an economically important model plant, such as tomato, under controlled laboratory conditions.

Acknowledgments

The authors are gratefully to Norma Angélica Martínez Gallardo for skillful technical assistance.

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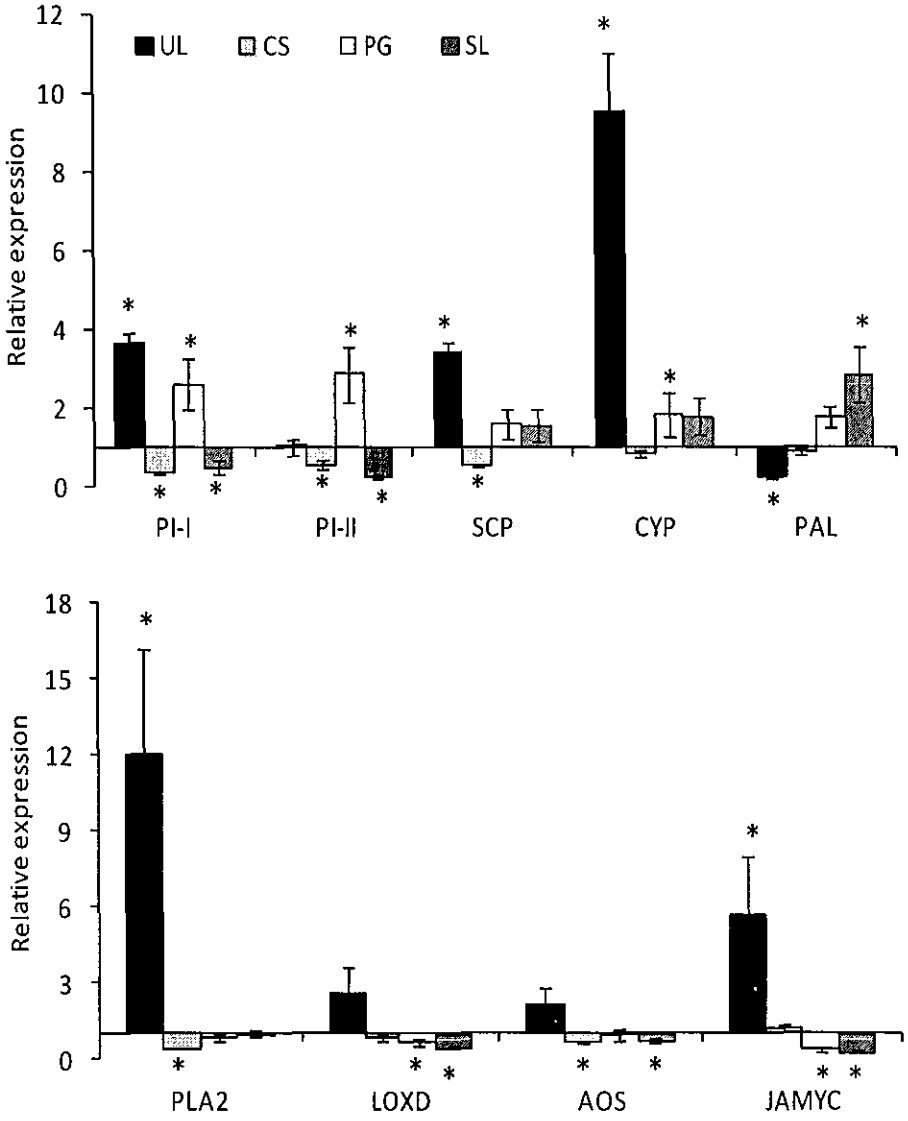

Fig 3 Expression profiles of defense-related genes in tomato plants in response to seaweed extracs from *Ulva lactuca* (UL), *Caulerpa sertularioides* (CS), *Sargassum liebmannii* (SL), and *Padina gymnospora* (PG). qRT-PCR analysis of genes encoding allene oxide synthase (*AOS*), lipoxygenase (*LOXD*), MYC transcription factor (*JAMYC2*), Phospholipase A2 (*PLA2*), wound-inducible carboxypeptidase (*SCP*), phenylalanine ammonia lyase (*PAL*), cysteine proteinase (*CYP2*), wound-induced proteinase inhibitor I (*PI-I*) and wound-induced proteinase inhibitor II (*PI-II*). Activities were measured at 24 h after treatment. All values were normalized to water-treated controls. Bars indicate mean ± SE (n = 3). Asterisks indicate significant differences ($P < 0.05$).

Table 1. Primers used for gene expression analysis by qRT PCR

Gene Symbol	Oligo Sequence Forward/Reverse	Amplicon Length (pb)	Accession Number
LOXD	GGTGTCATCGAGGCTTGC/ CGGATAAGGTCAGCTGGAAG	110	U37840
JAMYC2	TTGGCGTCAATTCAAATCTTC/ CCACGTCTCTCTAGCACCATC	182	AJ630505
PLA2	CTAATGCTGGCCTCTCCTTG/ CCCAGGGCATCCACTATACA	122	SGN-U569316
SCP	GTGGACCTGGTTGTTCAAG/ ATGGATTGTTGTGCAGAGAA	108	AF242849
AOS	GACGCATCATTCGAAATCAA / GCGTTTTCAGTTTCCGACCC	179	NM_001247904
PAL	GCTGAGCAACACAACCAAGA/ GCAAAGAGCCACGAGATAGG	114	M83314
CYP2	GGTCACACTCATGGTCACAA/ CATCTGATTTTGACTGGCG	180	Z14028
PI-I	GTCAAAGTTTGCTCACATCA/ TTCGCACATCAAGTTAGAGTC	133	K03290.1
PI-II	TGGCTGTTCACAAGGAAGTTAAT/ AATTGATGCATATGGGATTTAG	162	K03291.1
EFα	TTGGCCCTACTGGTTTGAC/ GATGATGACCTGGGCAGTG	200	X14449
TIP41	AAGCATGTAAATAGTGGCACAAAAT/ GACATGTTCACTTCCACAGTAAGGT	192	SGN-U321250

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VII. CONCLUSIONES

- La presente investigación demostró claramente que los extractos líquidos de algas marinas promueven el crecimiento e inducen defensa en las plantas de tomate.

- Los extractos de *U. lactuca* y *P. gymnospora* a 0.2 % de concentración incrementaron todos los parámetros de germinación (porcentaje de germinación, índice de germinación, energía de germinación, tiempo medio de germinación e índice de vigor de la plántula) y crecimiento de plántulas (longitud de plúmula y radícula) y plantas de tomate (longitud de brote y raíz).

- El pH y conductividad eléctrica de los extractos algales líquidos son responsables en gran medida de la germinación y crecimiento de plántulas de tomate (altas concentraciones de los extractos inhiben el crecimiento y desarrollo de plántulas de tomate).

- Los minerales y otros compuestos como hormonas de crecimiento presentes en los extractos de algas marinas, los hacen una opción alternativa de biofertilizantes o bioestimulantes.

- Todos los extractos etanólicos extraídos de las algas marinas a una concentración de 0.1 mg mL^{-1} inducen protección en plantas de tomate contra el hongo necrotrófico *Alternaria solani*

- Los extractos etanólicos *U. lactuca* y *P. gymnospora* inducen resistencia en las plantas a través de la activación de enzimas y expresión de genes relacionados con defensa, donde se observó una resistencia sistémica inducida vía ácido jasmónico/sistemina, en respuesta a heridas.

- Los inductores (carbohidratos) presentes en las algas marinas son los responsables de inducir defensa en las plantas de tomate.

VIII. RECOMENDACIONES

- Se recomienda emplear combinaciones de los tratamientos aplicados (al sustrato y foliar) al mismo tiempo, ya que podría ser un método de aplicación más efectivo.

- Realizar estudios, para evaluar el efecto de estos extractos en campo, sobre cultivos agrícolas económicamente importantes.

- También es necesario llevar a cabo estudios de tipo químicos en los extractos algales, para determinar sustancias o compuestos (hormonas) responsables del crecimiento y compuestos (carbohidratos) que funcionan como inductores de defensa de las plantas.

Una vez realizadas las investigaciones necesarias se puede determinar el potencial comercial de la *U. lactuca* y *P. gymnospora* como bioestimulantes, fertilizantes o inductores de defensa; y pueden ser promovidos como productos ecológicos, sin causar daño al medio ambiente.

IX. LITERATURA CITADA

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